Monitoring Protocols for the National Park Service North Atlantic Coastal Parks: Ecosystem Indicators of Estuarine Eutrophication

Version 1.0

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1 PROTOCOL NARRATIVE

1.1 Background and Objectives

1.1.1 *Introduction*

Nutrient enrichment of the coastal zone is a worldwide consequence of human population growth. Land clearing, fertilizer production and application, discharge of sewage and septic systems, and fossil fuel combustion have accelerated nitrogen and phosphorus loading to coastal ecosystems since the 1950's (Nixon 1995, Cloern 2001). Estuaries in the northeastern US are particularly threatened by human disturbances within the densely populated coastal zone (Roman et al. 2000). The Northeast (from Maine to Maryland) currently accounts for about one third of the coastal population of the entire United States (NOAA 1998). The population density of this narrow coastal fringe is more than double that of any other region of the country, and it continues to grow. The consequent residential, agricultural, and urban expansion will result in a continued increase in anthropogenic nutrient loading to the region's coastal zone. Estuaries can generally assimilate some degree of enrichment without major ecological ramifications, but excessive nutrient inputs typically lead to dense blooms of phytoplankton and fast-growing macroalgae, loss of seagrasses, and decreased oxygen availability in sediments and bottom waters (Valiela et al. 1992, Nixon 1995, Borum 1996, Bricker et al. 1999). Ultimately, cascading effects include changes in the species composition and abundance of invertebrates, decline in fish and wildlife habitat value, and the collapse of fin- and shellfish stocks.

National Park units along the North Atlantic coast protect a total of about 1,891 square kilometers between Virginia and Maine. Approximately one quarter of this land area is submerged (NPS 2000a). These estuaries, bays, and lagoons serve as islands of relatively pristine aquatic habitat within the northeastern urban corridor. The North Atlantic coastal parks are dependent on high-quality aquatic resources to sustain the complex estuarine and near shore ecosystems they represent. Diverse threats to NPS estuaries exist, including natural disturbances (e.g. storms, sea level rise), direct impacts of human activities (e.g. fishing, boating, dock construction), indirect effects of watershed development, and disasters (e.g. oil and toxic spills.) Of these, park managers have repeatedly identified threats to coastal water quality as one of their highest priority issues (PWRC 1999). Much of the watershed area of NPS coastal ecosystems lies outside protective park boundaries and is subject to intense developmental pressures. Therefore, there is great potential for human alterations of coastal watersheds to result in increased nutrient loading to park waters. Protecting the ecological integrity of park estuaries depends on implementing a scientifically-based monitoring program that is capable of diagnosing local causes of nutrient enrichment, detecting changes in nutrient loads, and determining if nutrient inputs are near to exceeding thresholds that would result in shifts in ecosystem structure and function (cf. NRC 2000).

The NPS Northeast Coastal and Barrier Network consists of eight parks from Massachusetts to Virginia (Figure 1). The four largest parks include extensive estuarine habitat (Assateague Island National Seashore, Maryland/Virginia; Cape Cod National Seashore, Massachusetts; Gateway National Recreation Area, New York/New Jersey; Fire Island National Seashore, New York). Colonial National Historic Park, Virginia, is a park of almost 8000 ha with a moderate amount of estuarine shoreline, and two small parks (Sagamore Hill National Historic Site, New York; George Washington Birthplace National Monument, Virginia) also include short stretches

of estuarine shoreline. The last of the network parks, Thomas Stone National Historic Site, neither contains nor directly abuts any estuarine resources. In addition, two parks within the adjacent Northeast Temperate Network also include extensive estuarine habitat (Acadia National Park, Maine; Boston Harbor Islands, Massachusetts). Collectively, these park units represent a wide range of sizes (33 ha to almost 20,000 ha), latitudes (37°11.3'N to 44°25.6'N or more than 800 km of latitude), watershed geologies (shallow soils overlying granite bedrock vs. thick sandy glacial deposits), tidal range (micro-tidal to over 3m), and fresh water sources (surface water vs. ground water; Roman et al. 2000). Estuaries within these parks share fundamental characteristics, however, including temperate zone flora and fauna and the threat of nutrient enrichment as a primary management concern (Roman et al. 2000). Their broad similarities are the basis for development of a uniform regional protocol for monitoring estuarine nutrient enrichment within the nine park units of the Northeast Coastal and Barrier Network and the Northeast Temperate Network that contain estuarine resources.

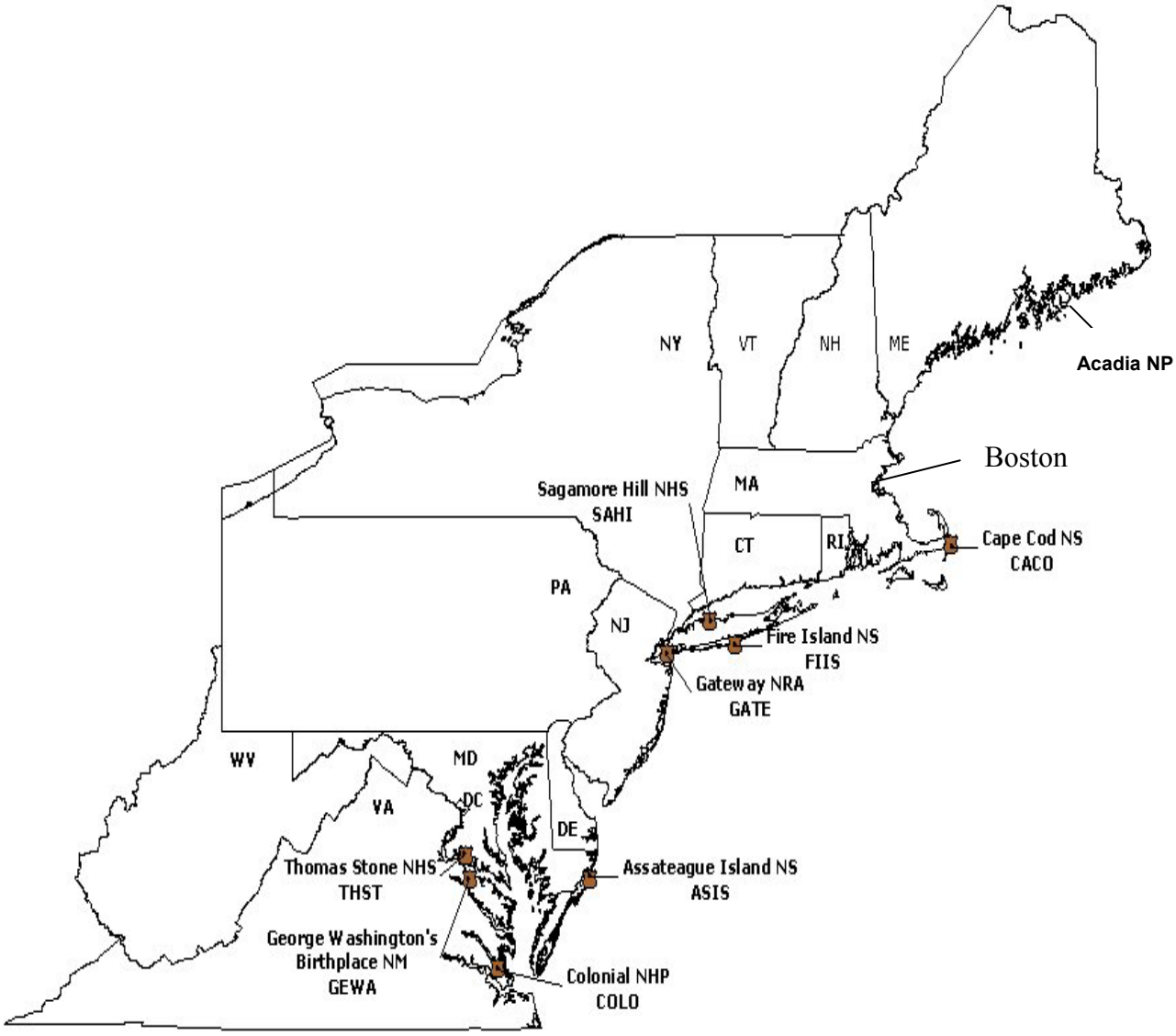

Figure 1. North Atlantic Coastal National Parks

1.1.2 *Monitoring Objectives*

The overall goals of estuarine nutrient monitoring are to identify the sources and consequences of nutrient inputs to park waters to assist in protecting and managing estuarine habitat. The purpose of this protocol is to detect and predict the consequences of estuarine nutrient enrichment; a separate protocol focuses on identifying the sources of nutrients entering park estuaries. The protocol described here addresses the following specific monitoring objectives:

Objective 1: Determine if nutrient loads to Park estuaries are increasing;

Objective 2: Determine if estuarine resources are changing in response to nutrient inputs.

Vital signs monitoring is based on indicators of estuarine responses to nutrient enrichment. The remainder of this document describes the development of the monitoring protocol, justification for vital signs selection, and approaches and standard operating procedures for implementation throughout North Atlantic coastal parks.

1.1.3 *Historical Development of the Monitoring Protocol*

A conceptual model linking human activities, nutrient loading, and estuarine ecosystem responses provided a framework for identifying variables to address the monitoring objectives (Figure 2). This model was adopted during development of the prototype monitoring protocol at Cape Cod National Seashore as a result of workshop discussions among scientists and NPS natural resource management professionals (Roman and Barrett 1999). The model helps identify key ecosystem threats (agents of change) and responses to consider during the process of variable selection. Although this particular graphic was developed for Cape Cod estuaries, the relationships depicted in the conceptual model are applicable to issues of estuarine nutrient enrichment worldwide (e.g., see similar conceptual models in Sand-Jensen and Borum 1991, Dennison et al. 1993, Batiuk et al. 2000, Cloern 2001).

Initially, an exhaustive list of variables exhibiting responses to estuarine nutrient enrichment was drawn from diverse sources. These potential vital signs were identified through technical workshops and meetings (Table 1), consideration of estuarine characteristics at North Atlantic coastal parks, and analysis of existing monitoring programs with relevance to park estuaries (Kopp et al. 2002). Potential vital signs were then evaluated in terms of the established characteristics of effective monitoring variables (Table 2). The most effective monitoring programs include variables that span levels of ecological organization (organisms to landscapes), relationships (causes of and responses to stress) and complexity (structure, function, and composition; Dale and Beyeler 2001). Consequently, each variable was evaluated in terms of its relative contribution to a collective suite, with the goal of including representatives of different scales, trophic levels, and relationships to nutrient enrichment.

The analysis of existing monitoring programs revealed many sources of data relevant to nutrient enrichment of park estuaries (Kopp et al. 2002). Information on types, quantity, and quality of existing data available for immediate use by the parks, or available to be leveraged with additional park effort, helped to identify potentially cost-effective variables for NPS Vital Signs monitoring. The efficiency associated with adopting uniform approaches for regional and national estuarine sampling across NPS programs and those of other federal agencies also guided variable selection. Potential variables were evaluated for consistency with two NPS programs also under development (national water quality monitoring in marine/estuarine waters, Roman et al. 2003; and water quality inventory protocols for estuarine/marine systems, Berounsky in

prep.), with the System-wide Monitoring Program of the National Estuarine Research Reserves (Sanger et al. 2002; http://nerrs.noaa.gov/Monitoring/welcome.html), and with the long-standing Environmental Monitoring and Assessment Program - National Coastal Assessment of the US Environmental Protection Agency (Jackson et al. 2000, US EPA 2001a; http://www.epa.gov/emap/nca/index.html). Thus the final list of candidate indicators for this protocol was influenced by both scientific and practical considerations.

The vital signs selected for network-wide monitoring of estuarine responses to nutrient enrichment are listed in Table 3, cross referenced by the Standard Operating Procedures relevant for their implementation. These variables are well justified scientifically, and collection of data for the entire suite is feasible from both practical and economic perspectives.

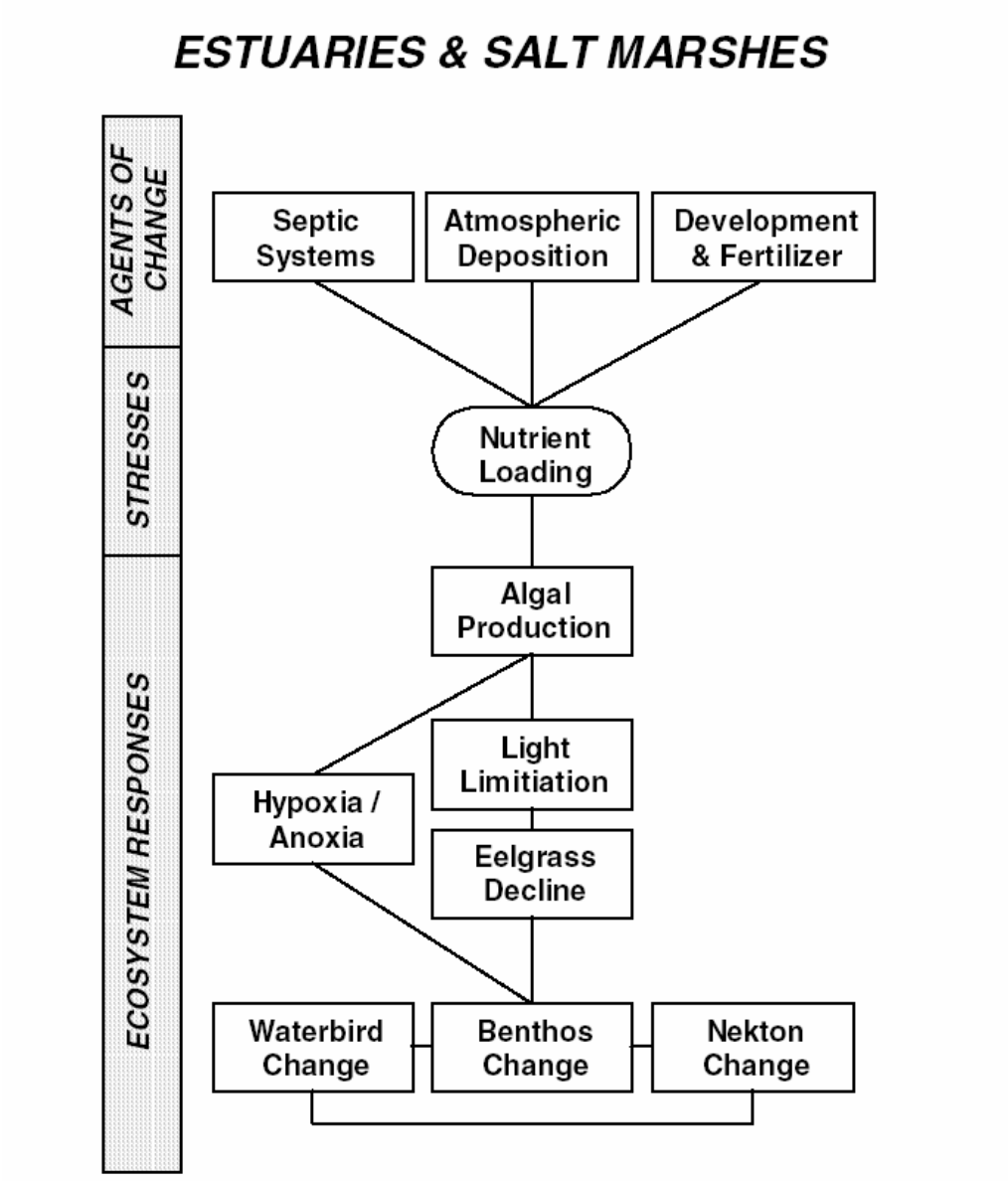

Figure 2. Conceptual model of estuarine ecosystem agents of change, nutrient load, and ecosystem response developed for the Cape Cod Prototype (from Roman and Barrett 1999).

Table 1. Technical workshops and work group meetings guiding variable selection process.

Meeting	Date	Focus	Participants
Coastal and Barrier Network Scoping Workshop, Water Quality Workgroup	April 13-14, 2000	Identification of monitoring questions Identification and ranking of many potential monitoring variables	Hilary Neckles – USGS John Portnoy – CACO Charles Rafkind – COLO Scott Gurney – SAHI Kirk Havens – VIMS Norm Rubinstein – USEPA Mark Ringenary – GATE Gary Rosenlieb – NPS WRD Rijk Morawe – GEWA/THST Brian Sturgis – ASIS
Coastal and Barrier Network, Estuarine Nutrient Enrichment Workgroup	February 12, 2001	Designed approach for selection of monitoring variables	Hilary Neckles – USGS Charles Roman – USGS Scott Nixon – URI Norm Rubinstein – USEPA Jim Latimer – USEPA Veronica Berounsky – URI Brian Sturgis – ASIS
Coastal and Barrier Network, Estuarine Nutrient Enrichment Workgroup	March 18, 2002	Final selection of monitoring variables for network-wide testing	Hilary Neckles – USGS Blaine Kopp – USGS Charles Roman – NPS NAC-CESU Scott Nixon – URI Barbara Nowicki – URI Gerald Pesch – USEPA Charles Strobel – USEPA Donald Cobb – USEPA

Acronyms: ASIS – Assateague Island National Seashore; CACO – Cape Cod National Seashore; COLO – Colonial National Historic Park; GATE – Gateway National Recreation Area; GEWA – George Washington Birthplace National Monument; NPS WRD – National Park Service Water Resources Division; NPS NAC-CESU – National Park Service North Atlantic Coast Cooperative Ecosystem Studies Unit; SAHI – Sagamore Hill National Historic Site; THST – Thomas Stone National Historic Site; URI – University of Rhode Island; US EPA – United States Environmental Protection Agency; URI – University of Rhode Island; USGS – United States Geological Survey; VIMS – Virginia Institute of Marine Science

Table 2. Characteristics of effective monitoring variables (after Jackson et al. 2000, NPS 2000b, Dale and Beyeler 2001, Kurtz et al. 2001, Fancy 2000).

Relevant to management concerns and ecological resources
Address monitoring questions of interest
Have known linkage to ecological function or critical resource of interest
Are at appropriate scale to answer specific monitoring questions
Are integrative in space and time, so that the full suite of variables provides assessment of entire system of interest
Applicable for use in a monitoring program
Are easy and practical to measure
Are non-destructive or low impact to measure without disturbing monitoring site
Are measurable using standard, well-documented methods
Generate data that are compatible with other systems
Are cost-effective to measure
Responsive to anthropogenic stresses
Have known sampling and measurement error
Have low natural variability
Have known variability in time and space
Are sensitive to anthropogenic stresses on the system or resource of interest, while having limited and documented sensitivity to other factors (i.e. to natural variation in ecosystem condition)
Interpretable and useful to environmental decision-making
Respond to stress in a predictable manner
Are anticipatory: signal impending change in ecosystem before substantial degradation occurs
Are linked to management decisions; predict changes that can be averted by management action, or document success of past actions
Have known or proposed thresholds of response that delineate acceptable from unacceptable ecological condition
Can be communicated to managers and the public

Table 3. Vital signs, measurements, and relevant Standard Operating Procedures for monitoring estuarine responses to nutrient enrichment.

Vital Sign	Measurement	Standard Operating Procedures
Estuarine Water Chemistry	Dissolved oxygen	SOP 1, 2, 3, 4, 5, 11, 12
	Temperature	SOP 1, 2, 3, 4, 5, 11, 12
	Salinity	SOP 1, 2, 3, 4, 5, 11, 12
Estuarine Water Quality	Chlorophyll *a*	SOP 1, 2, 3, 4, 5, 7, 11, 12
Estuarine Water Clarity	Attenuation of Photynthetically Available Radiation (PAR)	SOP 1, 2, 3, 4, 6, 11, 12
	Turbidity	SOP 1, 2, 3, 4, 5, 11, 12
Estuarine Sediment Organic Carbon	Percent organic carbon in surface sediments	SOP 1, 2, 5, 8, 11, 12
Seagrass Distribution	Submerged Aquatic Vegetation (SAV) bed size	SOP 9, 12
	SAV bed structure (cover class)	SOP 9, 12
	SAV bed location	SOP 9, 12
Seagrass Condition	Population-based measurements: Seagrass Density, Biomass, Canopy Height, Percent Cover, Seagrass Depth Limit	SOP 10, 12
	Shoot-based measurements: Epiphyte Cover, Wasting Index, Grazing	SOP 10, 12
	Ancillary measurements of on environmental suitability: Temperature, Salinity, Light Attenuation, and Sediment Parameters	SOP 10, 12

1.1.4 *Rationale for Vital Sign Selection*

1.1.4.1 *Estuarine Water Chemistry*

1.1.4.1.1 Measurement: Dissolved oxygen

Organic matter on the estuarine sediment surface and within the sediments is mineralized by microbial decomposers, a process that consumes oxygen. Consequently, as the pool of sedimentary organic matter increases in response to nutrient enrichment, intense benthic microbial metabolism can result in lowered concentrations of dissolved oxygen in bottom waters and a decrease in the depth of the oxic-anoxic interface within the sediments (Day et al. 1989, Cloern 2001). Ultimately, the shift to anaerobic benthic metabolism will stimulate sulfate reduction and cause an accumulation of hydrogen sulfide in pore waters (Herbert 1999, Cloern 2001). The increases in extent and duration of bottom water anoxia and concentration of toxic sulfide compounds with nutrient enrichment have obvious negative implications for benthic fauna. Dissolved oxygen concentrations below 2.0-5.0 mg/l cause declines in the diversity and abundance of estuarine fauna (NRC 2000). Therefore, use of dissolved oxygen as a monitoring variable provides indirect information on nutrient loads and direct information on threats to estuarine consumers. It is important to note, however, that changes in both sediment organic carbon and dissolved oxygen concentrations may also occur without changes in nutrient inputs, but may rather signal deposition and decomposition of allochthonous organic matter.

1.1.4.1.2 Measurement: Temperature and Salinity

Two of the most important physical characteristics of seawater are temperature and salinity. Although temperature and salinity data are not directly applicable to questions regarding estuarine nutrient enrichment, these variables are critical to interpreting the responses of other parameters. Temperature is a primary determinant of the rate of biological processes. Thus productivity and growth of phytoplankton (Goldman and Carpenter 1974) and SAV (Marsh et al. 1986, Bulthuis 1987), microbial metabolism (Christian et al. 1989), sediment oxygen demand (Portnoy 1991), nutrient remineralization (Nowicki and Nixon 1985), and faunal recruitment (Day et al. 1989) are all strongly influenced by temperature, and the annual patterns of primary producers and consumers at temperate latitudes are largely a function of seasonal temperature differences (Day et al. 1989). Temperature also controls rates of chemical reactions and solubility of gases in estuarine water, and so affects dissolved oxygen concentrations. Estuarine organisms differ in their osmoregulatory abilities and salinity tolerances, so salinity similarly exerts strong control on the distribution and abundance of estuarine flora (Verhoeven and van Vierssen 1978, Adams et al. 1992) and fauna (Nordby and Zedler 1991, Montague and Ley 1993, Engle et al. 1994).

It is clear that temperature and salinity may influence the response of other monitoring variables to nutrient enrichment. Therefore, given the broad temporal and spatial variation in estuarine temperature and salinity, these physical characteristics must form part of any estuarine monitoring program. Temperature and salinity data may also provide insight into sources of nutrient input. For example, groundwater is typically colder than ambient estuarine water in the summertime, so that temperature contrasts can be used to locate groundwater discharge zones (Portnoy et al. 1998). Estuarine salinity is strongly dependent on river and stream flow, so that pulses of surface water are often recorded as decreases in salinity. A final justification for long-

term monitoring of estuarine temperature and salinity is the potential for changes in these parameters to exacerbate effects of nutrient inputs in a changing global climate (cf. Short and Neckles 1999). For example, eelgrass (*Zostera marina*) becomes more susceptible to the negative impacts of algal epiphytes at high water temperatures (Neckles et al. 1993).

1.1.4.2 *Estuarine Water Quality*

1.1.4.2.1 Measurement: Chlorophyll a

Nutrient enrichment of coastal waters frequently stimulates phytoplankton production and results in increased phytoplankton biomass (Sand-Jensen and Borum 1991, Duarte 1995, Borum 1996). A strong linear relationship exists between input of dissolved inorganic nitrogen and phytoplankton production (when both are log transformed) in deep, phytoplankton-based marine systems (Nixon et al. 1996). Chlorophyll *a*, an indicator of phytoplankton biomass, shows a similar relationship in deep-water systems (Nixon 1992). Because of this, many national, regional, state, and local estuarine monitoring and assessment programs include measures of chlorophyll *a* concentration as an indicator of nutrient loading (e.g. Bricker 1999, Gibson et al. 2000, USEPA 2001b, and many programs summarized in Kopp et al. 2002). Several recent reviews of data from shallow coastal systems worldwide, however, have revealed considerable variation in the response of phytoplankton to nutrient enrichment (Borum 1996, Cloern 2001, Nixon et al. 2001), indicating that the linkage between nutrient increase and phytoplankton biomass is complex and system-dependent. It is now evident that many factors in addition to nutrient availability, including tidal energy, horizontal transport, optical properties, algal species composition, and benthic suspension feeders, exert strong control on phytoplankton population growth (Lucas et al. 1999, Cloern 2001, Nixon et al. 2001). These factors introduce non-linearity in the response of phytoplankton biomass to nutrient input (e.g. Borum and Sand-Jensen 1996), particularly in shallow, macrophyte-dominated estuaries.

Despite lack of a consistent linear response of phytoplankton to nutrient load, the fact that phytoplankton growth is indeed often nutrient-limited and the wide prevalence of chlorophyll *a* as a monitoring variable argues for the inclusion of chlorophyll *a* in a suite of estuarine vital signs. Although it is clear that low phytoplankton biomass does not necessarily indicate low rates of nutrient input, a trend of increasing biomass within a system may well correlate with increasing nutrient load. Long-term measurements of chlorophyll *a* in concert with other estuarine parameters may improve scientific understanding of the complex interactions among nutrient enrichment, inherent ecosystem attributes, and coastal ecosystem responses; this information should increase the sensitivity of the next generation of monitoring tools. Finally, since chlorophyll *a* data are widely available for estuaries nationwide (Bricker et al. 1999), this parameter offers a means to compare NPS coastal waters with many other systems.

1.1.4.3 *Estuarine Water Clarity*

1.1.4.3.1 Measurement: Attenuation of Photosynthetically Available Radiation (PAR) and Turbidity

The importance of submerged aquatic vegetation (SAV, including seagrasses and freshwater submerged macrophytes found in upper reaches of estuaries) to the ecological function and habitat value of shallow coastal systems is widely recognized, and estuarine protection and restoration goals frequently emphasize the goal of maintaining or increasing SAV abundance

(e.g. Chesapeake Bay Program 2000, many of the EPA National Estuary Programs profiled at http://www.epa.gov/owow/estuaries/list.htm). The principal environmental control on SAV productivity and distribution is light availability (e.g. Dennison and Alberte 1982, 1985; Dennison 1987, Duarte 1991), specifically the amount of photosynthetically available radiation (PAR, light between 400-700 nm) transmitted to plant leaves. A primary factor contributing to the attenuation of PAR through the water column is phytoplankton concentration (Figure 2; Dennison et al. 1993, Gallegos 1994, Krause-Jensen and Sand-Jensen 1998). Therefore, in systems showing increases in phytoplankton biomass with nutrient load, PAR attenuation is correlated with nutrient enrichment (Borum 1996). Measurement of average PAR attenuation thus provides information directly related to a living resource of management concern, and potentially related to nutrient enrichment. Attenuation of PAR also exerts control on phytoplankton growth, and is strongly influenced by concentrations of suspended inorganic material and colored dissolved organic matter (Gallegos 1994). Therefore, used in concert with chlorophyll *a* as a monitoring variable, information on PAR attenuation may offer insight into mechanisms causing changes in phytoplankton biomass. Other suspended particles (sediments, particulate organic material) also reduce light transmission through the water column (Kirk 1994). Measurement of the total turbidity, or the degree to which light traveling through the water is scattered and absorbed by suspended particles, concurrent with PAR attenuation can help determine the proportion of light reduction attributable to phytoplankton and, indirectly, to nutrient enrichment.

1.1.4.4 *Estuarine Sediment Organic Carbon*

Water column and benthic processes are closely coupled in shallow coastal systems, so responses to nutrient enrichment may be observed in the sedimentary environment (Herbert 1999, Cloern 2001). Some of the organic production stimulated by nutrient inputs may be exported to nearshore waters, but this is generally a small fraction of the total primary production. For example, 10-15% of the primary production in Narragansett Bay is exported from the system (Nixon et al. 1995), and some systems with high rates of primary production export less organic matter than they produce (Smith and Hollibaugh 1993). Thus the majority of increased production that is stimulated through nutrient enrichment is metabolized or stored within the system. Much of this authochthonous organic matter sinks to the benthos and contributes to the pool of sediment organic material. Thus, organic carbon in the sediments may increase with nutrient load. Striking evidence is found in sediment cores from Chesapeake Bay, where a doubling of organic carbon content over the past 80 years corresponds to a period of dramatic increases in nutrient load (Cornwell et al. 1996).

1.1.4.5 *Seagrass Distribution*

The correlation between increased nutrient loading and declines in SAV distribution has been documented for estuaries worldwide (reviewed by Sand-Jensen and Borum 1991, Duarte 1995, Harlin 1995). Experimental studies have confirmed the causal relationships linking nutrient input, increased algal production, and decreased macrophyte growth and survival (Neckles et al. 1993, Short et al. 1995, Taylor et al. 1995, Sturgis and Murray 1997). The primary mechanism for loss of SAV in response to increased nutrient load is attenuation of light by fast-growing phytoplankton, epiphytic microalgae, and free-floating macroalgae, resulting in reduced availability of light at macrophyte leaf surfaces (Figure 2; Sand-Jensen 1977, Bulthuis and Woelkerling 1983, Twilley et al. 1985, Sand-Jensen and Borum 1991). In Waquoit Bay,

Massachusetts, a significant negative relationship was found between eelgrass area and nitrogen loading (Short and Burdick 1996). On a larger scale, Nixon et al. (2001) examined 30 bays and estuaries, and found a lack of SAV in those that were highly enriched. Similarly, in an analysis of estuarine systems from around the world, Valiela and Cole (2002) demonstrated a positive relationship between nitrogen load and loss of seagrass cover. SAV-dominated estuaries are considered more sensitive to nutrient input than are estuaries dominated by plankton (NRC 2000), and restoration and protection of SAV habitat is often a primary management goal. Thus, the trend in SAV distribution over time offers an indicator of changes in nutrient load, while providing information directly applicable to critical habitat protection efforts. Data must be interpreted with caution, however, because factors other than light availability at leaf surfaces also exert significant control on SAV distribution, including substrate type, wave energy, current velocity, and contaminant exposure (Koch 2001).

1.1.4.6 *Seagrass Condition*

1.1.4.6.1 Population-based measurements: Seagrass Density, Biomass, Canopy Height, Percent Cover, Seagrass Depth Limit

The structure of seagrass meadows is a result of shoot recruitment, growth, and mortality, processes that frequently exhibit responses to nutrient enrichment. Seagrass characteristics that indicate changes in these processes can signal changes in the integrity of the seagrass bed. These population-based characteristics typically respond to environmental stress before an entire seagrass bed is lost or hypoxic conditions develop in the estuary, and thus provide more anticipatory indicators of changing ecosystem condition.

Decreases in seagrass shoot density occur when shoot recruitment and mortality rates combine to cause a negative net rate of population change. Eelgrass shoot density declines predictably with declining light availability (Short et al. 1995). Because the primary mechanism by which nutrient enrichment affects seagrasses is through increased algal shading, shoot density has been shown to decrease with enrichment of shallow experimental systems (Neckles et al. 1993, Short et al. 1995, Nixon et al. 2001), with abundance of macroalgae in field manipulations (Nelson and Lee 2001), and across large-scale gradients of increasing land-derived nutrient loads (Hauxwell et al. 2003). Dynamic characteristics such as leaf-area productivity (Durako 1994) and leaf growth rate (Nixon et al. 2001) also respond to nutrient loading, and are reflected by structural indicators such as plant biomass and canopy height. Percent cover of seagrass leaves integrates over shoot density and biomass and is easy to measure; in lower Chesapeake Bay, significant linear relationships were found between percent cover and both shoot density and biomass of eelgrass and widgeon grass (*Ruppia maritima*; Orth and Moore 1988). The maximum depth limit of seagrass is directly related to light penetration through the water column (Dennison 1987, Duarte 1991). A decrease in the maximum depth limit of a given seagrass bed thus indicates long-term reductions in light availability that could potentially result from increased nutrient loading; a negative relationship between total nitrogen concentration in the water column and the lower depth limit of eelgrass has indeed been documented for Danish coastal waters (Borum 1996). Because these characteristics are related ultimately to the amount of light reaching seagrass leaves, we would expect to see changes first in the deeper parts of existing seagrass beds (Sand-Jensen and Borum 1991, Borum 1996).

1.1.4.6.2 Shoot-based measurements: Epiphyte Cover, Wasting Index, Grazing

In many coastal environments, nutrient enrichment causes increased growth of seagrass epiphytes (algae, bacteria, fungi, and protozoans on leaf surfaces; e.g. Orth and van Montfrans 1984, Silberstein et al. 1986, Sand-Jensen and Borum 1991, Tomasko et al. 1996). Typically, below a certain threshold of nutrient input, invertebrate grazers limit epiphyte biomass accumulation (Neckles et al. 1993, Williams and Ruckelshaus 1993). Ultimately, however, high epiphyte loads will reduce light and carbon availability at leaf surfaces, with negative impacts on macrophyte productivity (Sand-Jensen 1977, San-Jensen and Borum 1984, 1991). Control of epiphyte growth by grazers and other environmental factors is complex. The nutrient load at which epiphyte growth outpaces grazing varies seasonally (Neckles et al. 1993) and among systems, and some seagrass systems respond to increased nutrient levels with proliferation of other algal forms (phytoplankton and macroalgae; Short et al. 1995, Taylor et al. 1995, Nixon et al. 2001). However, seagrass declines have been attributed to epiphytic overgrowth (e.g. Twilley et al. 1986, Cambridge et al. 1986). Thus, as well as monitoring estuarine phytoplankton abundance (via chlorophyll *a* concentration), it is prudent to also monitor seagrass epiphyte cover as an early indicator of nutrient enrichment.

There are many factors other than anthropogenic nutrient enrichment that impact seagrass ecosystems. Direct physical disturbance (e.g. dredging and fill activities associated with coastal construction, mechanical damage from fishing and boating practices, strong storms and hurricanes) causes obvious local impacts and can trigger significant seagrass declines (Short and Wylie-Echeverria 1996, Duarte 2002). Some other sources of large-scale declines are not as readily recognized. The eelgrass wasting disease results from infection by the marine slime mold *Labyrinthula zosterae*. Wasting disease led to catastrophic losses of eelgrass in Europe and North America in the 1930's (den Hartog 1987), and continues to cause scattered, local declines in western North Atlantic populations (Short et al. 1986, Burdick et al. 1993). The environmental factors triggering outbreaks of wasting disease are not completely understood (Duarte 2002), but characteristic symptoms of the disease have been identified and are easily monitored (Burdick et al. 1993). Eelgrass declines have also been reported in response to intense grazing activity (by limpets, Zimmerman et al. 1996; Canada geese, Rivers and Short 2004; green crabs, Garbary et al. 2004). Monitoring seagrass shoots for evidence of wasting disease and grazing can help distinguish among potential sources of disturbance in ascribing changes to nutrient enrichment.

1.1.4.6.3 Ancillary Data: Light Attenuation, Sediment Parameters, Temperature, and Salinity

Seagrass production, growth, and distribution are regulated strongly by light availability (Duarte 1991, Dennison et al. 1993) and sediment characteristics (Barko and Smart 1996, Koch 2001) and are mediated by temperature and salinity tolerances with respect to environmental conditions (Short and Neckles 1999, Hemminga and Duarte 2000). These physical habitat features can thus influence seagrass responses to nutrient enrichment. Information on the range of light attenuation, sediment properties, temperature, and salinity of seagrass habitat must be monitoring in conjunction with seagrass condition indicators to interpret potential effects of nutrient inputs on estuarine integrity.

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1.2 Sampling Design

1.2.1 *Introduction*

Monitoring responses to estuarine nutrient enrichment incorporates several spatial sampling designs to address questions at a hierarchy of scales. One vital sign, seagrass distribution, is measured completely within each park unit. Vital signs related to estuarine water quality and sediments are sampled using a probability design within entire park estuaries. For water quality vital signs, this spatial sampling is coupled with continuous monitoring at representative sites. The final vital sign, seagrass condition, is sampled using a probability design within selected seagrass beds. By combining small scale, coarse measurements of entire estuaries with large scale, high resolution measurements of selected areas, this mixture of sampling approaches and intensities enhances the likelihood of detecting changes in response to nutrient enrichment.

1.2.2 *Seagrass Distribution*

Measures of seagrass distribution include the size, location, and structure of beds of submerged aquatic vegetation (SAV) within each North Atlantic coastal park unit. Data are obtained by mapping all SAV beds within park boundaries from aerial photographs. Measurements are made by photointerpretation following national data standards for benthic habitat mapping (Finkbeiner et al. 2001). No spatial sampling design is implemented for this vital sign because the entire resource is mapped. SAV mapping occurs every five years.

1.2.3 *Estuarine Water Chemistry, Estuarine Water Quality, Estuarine Water Clarity, and Estuarine Sediment Organic Carbon*

Spatial sampling of estuarine water chemistry, water quality, water clarity, and sediment organic carbon uses a probability-based systematic survey design. The sampling framework for each park is a grid of tessellated hexagons that encompasses the estuarine area of interest. Grids contain 30 hexagons, and vital sign sampling occurs at a random location in each hexagon. The number of hexagons was determined based on the known spatial variability of vital sign measurements and the desired degree of change detection. The systematic survey of water-column measurements occurs weekly during a four-week summer index period each year, and the survey of sediment organic carbon occurs every five years. Because the survey is probability based, it allows inferences to be drawn regarding the status of entire park estuaries. In addition to this spatial survey, one station is established at each park for continuous monitoring of water chemistry, water quality, and water clarity measures throughout each index period. These stations are not part of the probability design. Stations are selected to be representative of the overall estuary. Continuous monitoring data are not used to make inferences to other locations beyond the sampling stations. However, estuarine vital signs are known to exhibit a high degree of temporal variability, with important events occurring more frequently than weekly sampling may detect. Thus the continuous data are valuable in interpreting the patterns observed through systematic sampling.

1.2.4 *Seagrass Condition*

Within-bed measures of seagrass condition, including percent cover, shoot density, canopy height, and areal biomass, are sampled using a cluster sampling design stratified by depth zone. Seagrass responses to nutrient enrichment are typically detected first at the deepest locations of existing beds (cf. Background and Objectives 1.1.4.6). Therefore, the sampling objective of seagrass condition monitoring is to compare trends in seagrass characteristics between shallow and deep locations of existing habitat. This requires a much smaller sampling investment than would be required to describe an entire seagrass bed. One 50-m transect is located within each of three depth zones (shallow, mid-depth, and deep) in a seagrass bed that is representative of park seagrass habitat. Twelve sampling locations are then randomly positioned along each transect. Sampling occurs within and adjacent to 0.25-m^2 plots, and plots are revisited at least annually; seasonal sampling (4 times per year) is advantageous if resources are available.

1.3 Field Methods

1.3.1 *Field Schedule and Preparations*

The monitoring described in this protocol is implemented annually during July-August in each North Atlantic Coastal Park. The ability to successfully complete the annual monitoring will hinge on thorough advance preparation. The most important preparation is ensuring that personnel are available and trained to accomplish the field work. The specific staffing model for conducting the monitoring will vary by locale, depending on the presence of ongoing park water quality monitoring programs and the proximity of academic or other institutions skilled in estuarine monitoring. As much as possible, the NPS Networks will collaborate with individual parks and enter into agreements with nearby institutions to conduct the monitoring (see SOP 2, Field Season Logistics). In these instances, preparations will involve establishing cooperative agreements and general training by the Network coordinator in implementing this protocol. For parks where neither of these options is logistically or economically feasible, monitoring may fall to the Network. Additional preparation would then involve hiring seasonal technicians.

Advance preparation for actual sampling are listed in SOP 2, Table 13. These include establishing continuous water-column monitoring stations (one per park, SOP 3); obtaining calibration training from YSI, Inc., for proper use of the YSI multi-parameter water quality monitor; obtaining factory re-calibration of LiCor light sensors, identifying contract laboratories for analysis of chlorophyll and sediment samples; obtaining any necessary safety training, including the DOI Motorboat Operator Certification Course and potentially SCUBA authorization; and identifying and establishing seagrass condition monitoring stations (SOP 10).

1.3.2 *Sampling Methods*

Estuarine water chemistry, estuarine water quality, and estuarine water clarity measurements are made using the YSI 6600 multi-parameter water quality monitor (sonde) for spatial surveys and the complementary YSI 6600 EDS (extended deployment

system) for continuous monitoring of dissolved oxygen, turbidity, chlorophyll *a*, salinity, and temperature (SOP 4, SOP 5). Attenuation of Photosynthetically Available Radiation (PAR) is similarly monitored discretely (SOP 6) and continuously (SOP 4) using LiCor PAR sensors. Spatial surveys and continuous monitoring of chlorophyll *a* using the YSI sonde is supplemented with collection of near-surface water samples that are filtered through glass fiber filters and analyzed for chlorophyll *a* (SOP 7). Every five years, the spatial survey of water-column variables is supplemented with collection of samples for sediment organic carbon determination (SOP 8). Seagrass condition is monitored annually through measures of seagrass population-, shoot-, and habitat-based parameters (SOP10). Details of all sampling methods are provided in the accompanying Standard Operating Procedures.

1.4 Data Management

1.5 Analysis and Reporting

1.6 Personnel and Operational Requirements

1.6.1 *Staffing models for water quality monitoring*

There are three different staffing models that have been considered for implementing this protocol, each with its own unique logistical requirements. Experience gained during the feasibility test phase of protocol development suggests that a field crew of 2-3 people (depending upon the complexity of boat logistics) can manage water quality monitoring at two parks. If staffing for individual parks, a single skilled field technician can manage the water quality program provided that s/he has access to part time assistance in the form of a boat operator and occasional field assistants. The three most feasible staffing models are as follows.

1) Monitoring Conducted by the Individual Park –
Several North Atlantic Coastal Parks have well-established monitoring programs for estuarine water quality. For these parks, collaboration will likely be mutually beneficial to both the Networks and the Parks. The nature of any cooperative agreement must be negotiated, but a likely model is for the Networks to provide funding and/or seasonal staff to offset the additional cost of the Network monitoring protocol. This is a very attractive model because it doesn't require a large travel budget, it builds capabilities at the individual parks, and it leverages existing resources.

2) Monitoring Conducted by an Academic Institution –
For some of the parks, it may be advantageous for the Network to contract out the monitoring work to an academic institution. Most of the NACPs are in reasonably close proximity to an academic institution with investigators and a student workforce capable of implementing the protocol. An advantage of this model is that it allows the networks to take advantage of existing resources (boats, laboratories) without having to invest additional capital. Universities may also be able to use personnel more efficiently through specialization (e.g. dedicated boat drivers may be paid by the hour rather than having to train all field crew; chlorophyll grab samples may be filtered and run on site by skilled technicians rather than filtered by field crews then express shipped to labs).

3) Monitoring Conducted by the Network –

When neither of the above models is appropriate or available, or if they are logistically or economically infeasible, then monitoring work will fall to the Networks. There are some disadvantages to this model however. The logistics of managing multiple parks from a remote site is difficult, and additional travel costs would be incurred for temporary duty assignments while working at the various parks. One advantage to this model is that the network would maintain more control in the year-to-year consistency of the program; however, this goal can also be met through network run training services (see 1.6.3). Any of these staffing models will work, but individual parks may be more suited to one model over another (Table 4).

Table 4. Recommended staffing approaches for the water quality component of estuarine vital signs monitoring

Park Unit(s)	Recommended Staffing Approaches
ACAD	Although ACAD does not currently have an estuarine monitoring program, it does maintain a staff of natural resource scientists who conduct other monitoring activities. If ACAD is interested in expanding its program to include this protocol, NETN should pursue this as the most desirable option.
ASIS	ASIS already has a very strong estuarine water quality monitoring program. NCBN should pursue an agreement with the park to supplement its existing monitoring program so that Network information needs are also met.
BOHA	This park is already monitored by the Massachusetts Water Resources Authority, but not to the extent recommended by this protocol. Because of more complex logistics and boat requirements, NETN should pursue a cooperative agreement with one of the Boston-area universities.
CACO	Although CACO does not currently have an estuarine monitoring program, it does maintain a staff of natural resource scientists who conduct other monitoring activities. If the park is interested in expanding its program to include this protocol, NCBN should pursue this as the most desirable option.
COLO+GEWA	For these parks it would be advantageous for NCBN to contract out the monitoring work to an academic institution such as the Virginia Institute of Marine Science (VIMS). VIMS is preeminent in it expertise in estuarine monitoring, and investigators at VIMS already conduct very similar monitoring with the same or similar equipment in the nearby vicinity of COLO and GEWA.
GATE	GATE already has a very strong estuarine water quality monitoring program. NCBN should pursue an agreement with the park to supplement its existing monitoring program so that Network information needs are also met.
FIIS+SAHI	SAHI requires only the continuous monitoring component of this protocol, so it may best be accommodated by grouping it with FIIS. None of the logistical models are clearly better than the others for this park, so a staffing mechanism should be decided upon based upon NCBN and park interests.

1.6.2 *Annual Workload and Field Schedule*

Exact staffing needs for the field component of estuarine Vital Signs monitoring will depend on which staffing models are selected and will require negotiation with the parks and university or contract investigators. Estimates of the personnel requirements for field work associated with water quality and sediment organic carbon monitoring are listed in Table 5. If using the recommended staffing models, this will include a combination of personnel from individual parks, cooperators, and the networks. Estimates are included here for all parks as a basis for negotiating agreements with parks and cooperators.

Seagrass monitoring will only be implemented at the 4 parks with significant seagrass resources: ACAD, ASIS, CACO and FIIS. A minimum of 3-4 people are required for the 3 -4 days of fieldwork associated with seagrass condition monitoring (for two sites per park). This number assumes the sites are already set up and intact. Initial set-up will require six people over two days for each monitoring site. Seasonal monitoring is recommended for the first year of the program, then once a year (July) thereafter. Familiarity with a site and year-to-year continuity is very important for the successful implementation of this Vital Sign. Ideally the work should be staffed by an investigator with a professional interest in staying involved over a long term. Since the timing of seagrass condition monitoring depends upon spring low tides occurring in July, it will require separate crews for each NACP where it is implemented (although a single investigator might reasonably manage more than one crew).

Table 5. Estimated staffing requirements for the summer fieldwork associated with spatial and continuous monitoring of water quality and sediment organic carbon.

Park	No. of field personnel (Park, Cooperator, or Network)	Duration	Total Pay Periods
ACAD	2	14	14
BOHA	2	14	14
NETN Totals	**4**	**-**	**28**
CACO	2	14	14
FIIS+SAHI	2	14	14
GATE	1.5	14	10.5
GEWA+COLO	2.5	14	17.5
ASIS	1.5	14	10.5
NCBN Totals	**9.5**	**-**	**66.5**

The ability to successfully complete the index-period monitoring will hinge upon thorough preparation in advance of the field season. In addition to staffing the program and establishing contracts for services, many of the SOPs in this protocol require preparatory steps that must be completed well in advance of the index period or monitoring event. Field and laboratory equipment used for each SOP must be thoroughly inspected, tested and repaired or replaced as necessary; expendable supplies must be stocked; and field crews must receive safety and protocol training. For all three of the logistical models, the Network must play an active role in methods training for field personnel to ensure the protocol is implemented properly and to assure data consistency and quality. Notable preparations that may require additional lead time are listed in Table 6. The general timing of protocol activities related to water quality, benthic organic carbon, and seagrass condition are outlined in Table 7.

Table 6. Preparations for index-period monitoring requiring exceptional lead time and/or advanced planning.

SOP #	SOP Topic	Advance Preparations
3	Continuous logging Station	- If installation of continuous monitoring station is to be permanent, obtain applicable PAToN permit from US Coast Guard. - If installation is to be temporary (<6 months) then make appropriate notifications to US Coast Guard. - Construct and install support structure and mounting brackets in advance of index period.
4&5	YSI-water quality sonde	- Obtain calibration training from YSI, Inc. - If purchasing new equipment, note that the YSI for SOP 4 is factory customized and may require additional lead time to fill the order.
4&6	LiCor- PAR equipment	- Obtain factory re-calibration of PAR sensors. Calibrate biennially for sensors used only in spatial monitoring and annually for sensors used in continuous monitoring.
7	Discrete chlorophyll-*a*	- Identify an analytical laboratory to analyze discrete grab samples for chlorophyll-*a*. Enter into a contract agreement that includes specific on all required data quality assurance measures.
8	Sediment TOC	- Identify laboratory to analyze sediment samples for TOC. Enter into contract agreement that includes specifics on all required data quality assurance measures.
3-8, 10	Motorboat Operations	- -Train and certify boat operators with a DOI approved Motorboat Operator Certification Course (valid for 5 years).
10	Seagrass Condition	- Identify monitoring site and establish monitoring transects - Identify laboratory to analyze sediment samples.

Table 7. Schedule of training and field activities.

June:	Safety training Motorboat training YSI and LiCor instrument training General training for water quality and sediment OC monitoring Installation of a support structure to hold a continuous water quality sonde
July:	Reconnaissance and familiarization with the field setting Seagrass condition training Seagrass condition monitoring Initiation of continuous monitoring at the logging station Initiation of weekly spatial water quality cruises
August:	Continuation of weekly survey cruises Continuation of continuous monitoring at the logging station Completion of index period monitoring
Late August – Early September	Removal of temporary logging station fixtures Organize and review all data sheets and digital data files Clean, service, and store all equipment

1.6.3 *Personnel Training*

Staffing of this protocol requires a field team with a broad range of talents. Some of the required skills can be taught as part of the annual training procedures, but at least one member of each team must already have very strong skills in seamanship and small boat handling. At parks where the monitoring will be conducted by the National Park Service (whether the park or the network), a boat operator must be available who can pass a DOI Motorboat Operator Certification Course (MOCC).

The Standard Operating Procedures included in this document are intended to be a comprehensive description of the methods associated with Vital Signs monitoring in North Atlantic Coastal Park (NACP) estuaries. They are not, however, intended to be a training manual for field or laboratory personnel. Whether staffed by NPS employees or independent contractors, Vital Signs personnel will need additional training and support in order to successfully and safely complete the work described herein. Safety, in particular, must always be the highest priority. All NPS employees doing field or laboratory work for this protocol should be trained in cardio-pulmonary resuscitation (CPR) and basic first aid. Employees working in laboratory settings must receive safety training to do so even if their immediate work tasks involve no hazardous substances (dry ice is a safety concern for both lab and field personnel however). Likewise, all US Department of Interior employees who operate motorboats must receive training and safety certification to do so (DOI Motorboat Operator Certification Course). A basic assumption of this program is that all DOI and NPS safety guidelines will be followed while completing the work. Regardless of the methods provided in this protocol and regardless of existing DOI and NPS safety guidance (or lack thereof), it is the job of each employee to work safely. Should the methods in this document contradict your own sense of safety, then it is your obligation to discuss this concern with Network supervisors.

In addition to the safety training, field crews will need general training on protocol implementation for conducting all water column, sediment, and seagrass monitoring. Additionally, personnel will require detailed training on calibration, programming and use of the YSI and LiCor instruments. Training is an area of protocol implementation where the networks can be most positive influence on data quality and on the constancy of the implementation among parks and over time. Rather than attempting to staff the actual field program, the networks are advised to emphasize their role as trainers. This role should be assumed for both park-lead monitoring (model 1) and monitoring outsourced to academic institutions (model 2).

1.6.4 *Equipment Needs and Startup Costs*

There are significant equipment needs for this protocol, and an up-front purchase of it all may be prohibitively expensive. There are, however, some reasonable mechanisms to minimize the initial investment and to better distribute these costs over time. Major equipment costs are associated with the monitoring procedures addressing the Vital Signs of 1) Estuarine Water Chemistry, 2) Estuarine Water Quality, and 3) Estuarine Water Clarity. These are covered by SOPs 3-6 and involve the used of sophisticated instrumentation. Each NACP where this protocol is implemented will need two YSI Multi-Parameter Water Quality Sondes with a suite of sensors. One of these is used for spatial survey work. The other requires a factory modification to accept LiCor PAR sensors and is used for continuous water quality monitoring. Anticipated prices for the components of these instruments (Table 8) add to a total cost of over $30,000 per park. This does not take into account several mitigating factors.

1- ASIS already conducts continuous monitoring at three locations in the park, and has an established spatial monitoring program. NCBN is unlikely to need a full set of instrumentation for this park, and resources may be better spent by subsidizing costs for park-owned instrumentation.

2- If the NCBN chooses to contract out the work at COLO and GEWA, it may be feasible to rent instrumentation from the Virginia Institute of Marine Science and/or the Virginia NOAA National Estuarine Research Reserve as part of the agreement.

3- SAHI does not require dedicated instruments for spatial monitoring since those components of the protocol is omitted for the park. Additional equipment is needed only for spot checks during routing servicing of the continuous sensor array, and this could be borrowed from one of the other NY parks.

4- It is feasible that instrumentation for the spatial surveys could be shared between two parks provided they are in reasonably close proximity and can coordinate fieldwork.

5- The NCBN has already invested almost $50,000 in this instrumentation for the development stages of the protocol (including four YSI 6600EDS with sensors, one 650MDS, four Li1400, and seven underwater PAR sensors and cables).

6- It is possible to rent these instruments on a short-term basis from companies such as US Environmental Rental http://www.gonasco.com/us_env_.htm and Frondriest Environmental http://www.fondriest.com/.

These factors will allow the networks to implement this protocol using state of the art instrumentation without assigning a disproportionate amount of the budget toward equipment.

Table 8. Instrumentation requirements and costs for estuarine water quality monitoring. This estimate is based upon the assumption that each park requires a full suite of instrumentation.

	Equipment Description	Quantity/ Park	Unit Cost	Extend
A	YSI 6600 EDS Multi-Parameter Water Quality Logger w/ 6560 Temperature-Conductivity Probe and Upgrade to accept input from LiCor PAR sensors (including wiper motors)	1	$8,665	$8,665
B	Standard YSI 6600, 6920,or 6600EDS Multi-Parameter Water Quality Logger w/ 6560 Temperature-Conductivity Probe	1	$4,770-$5,660	$5,690
C	YSI 6562 6-Series DO Probe Kit	2	$500	$1,000
D	YSI 6026 Turbidity Probe	2	$1,340	$2,680
E	YSI 6025 Fluorescence Chlorophyll Probe Assembly	2	$2,630	$5,260
F	YSI 650 Multiparameter Display System w/ full memory and barometer	1	$2,250	$2,250
G	YSI 6091 Field Cable	1	$390	$390
H	YSI 6067B Dry Calibration Cable	1	$170	$170
J	LiCor Li-1400 Datalogger	1	$1,350	$1,350
K	LiCor Li-192SA Underwater Quantum Sensor	3	$525	$1,575
Ll	LiCor Li-190SA Quantum Sensor	1	$315	$315
M	LiCor Lowering Frame	1	$115	$115
N	LiCor Mounting & Leveling Fixture	1	$41	$41
O	LiCor Underwater Cable	1	$275	$275
P	WAAS Enabled GPS Unit	1	$275	$275
Q	YSI 6115 GPS Cable	1	$100 est	$100
	Total Instrumentation Cost Per Park			**$30,151.00**

1.7 Version Control Procedures

2 STANDARD OPERATING PROCECURES (SOPs)

2.1 SOP 1 – Technical Information on Spatial Sampling Designs for Estuarine Water Chemistry, Estuarine Water Quality, Estuarine Water Clarity, and Estuarine Sediment Organic Carbon

2.1.1 *Introduction*

This SOP describes the methods used to select sampling locations for measures of estuarine water chemistry, water quality, water clarity, and sediment organic carbon within the North Atlantic Coastal Parks (NACP). The sampling design includes a probability survey of estuaries at each park coupled with continuous monitoring at representative sites. Sampling is conducted annually during an index period consisting of four consecutive weeks during July and August. This is the portion of the year when estuarine vital signs are expected to show the greatest response to nutrient over-enrichment.

Probability sampling ensures that samples are unbiased and can be used to make inferences about areas within the estuary that are not sampled. In probability designs, every element in the area or population of interest has a known chance of being sampled, and sampling includes a random component. The probability survey design adopted for NACP estuaries uses a grid of tessellated hexagons as the basis for sample site selection. Specific sampling locations are selected randomly within each hexagon. This design was developed by the USEPA Environmental Monitoring and Assessment Program in the early 1990s, and first applied to estuaries in the Louisianan (Summers et al. 1993) and Virginian (Strobel et al. 1994) provinces. It has since been adopted by the EPA National Coastal Assessment (U.S. EPA. 2001). The hexagonal sampling grid results in a set of samples that is balanced spatially over the entire estuary of interest. This approach thus combines the benefits of a systematic design with the statistical advantages of random sampling. Because this sampling protocol is consistent with the EPA National Coastal Assessment, the condition of NACP estuaries can be evaluated within the context of other estuaries in the region and NACP Vital Signs data can contribute to overall regional assessments.

For this protocol, the estuarine area of interest within each park is encompassed within 30 tessellated hexagons. The sample size is based upon experiences gained by the EPA National Coastal Assessment, and is corroborated by an evaluation of data variance at CACO and Network change-detection needs (Table 9). For binomial variables (as are used to determine the proportion of park estuaries that meets designated criteria for estuarine condition) the power of the design is a function of the rarity of the event as well as the sample size; for continuous variables (as are used to estimate the mean values of vital signs in park estuaries) it is driven by sample size and metric variance. For both, each additional increment in sampling effort produces a diminishing return in change-detection capability. Thirty spatial elements is selected as a compromise where the Network will have enough information to track long-term changes at its estuaries, while still being logistically and economically feasible to accomplish.

Table 9 Percent change that can be detected between two assessments of condition using various sample sizes (N) of binomial and continuous variables. All the values are based upon a significance level (i.e. P-value, or the likelihood that an observed difference is due to chance) of 0.05 and a 0.8 probability of detection (i.e. statistical power, or the likelihood of detecting a real change). Estimates for binomial data use the normal approximation and assume infinite population size. The smallest detectible change is calculated for both rare (P_1=0.01) and common (P_1=0.5) occurrences (where P_1 represents the proportion of a sample meeting a designated threshold in the first of two assessments. Estimates for continuous data are based upon the formula of Sokal & Rohlof (1981), and are shown for data with moderate and high coefficients of variation (V). These are typical of what is seen for the percent saturation of dissolved oxygen (V=20%) and the concentration of phytoplankton chlorophyll-*a* (V=50%) in Pleasant Bay at Cape Cod National Seashore (PBRMA 2004).

	binomial variables		continuous variables	
N	P_1=0.01	P_1=0.5	V=20%	V=50%
15	36%	41%	21%	53%
30	24%	32%	15%	37%
50	16%	26%	11%	28%

A randomly generated point within the estuarine domain of each hexagon is selected for sampling. Within the majority of the hexagons, sampling locations are repositioned randomly each year. A subset of the hexagons (generally 20%) is designated for trend monitoring. Trend stations are initially positioned randomly within their respective hexagons, but they are not repositioned from year to year; they are sampled each week of the index period, and sampling is repeated at the same stations for each year of monitoring. Parks with established monitoring programs are able to use their existing stations as trend stations for the hexagons in which they fall when certain conditions are met. Samples from every hexagon, including the random-repositioned and the trend stations, contribute annually to an overall assessment of estuarine condition. In addition, samples from the trend stations allow intra-seasonal and interannual comparisons of estuarine condition at select locations with a high degree of precision. Continuous monitoring stations (generally one per park) are located in areas considered to be representative of the majority of the estuarine environment at each park. Continuous data are used to identify temporal variability in estuarine vital signs that is not captured by weekly trend monitoring. Although continuous stations do not contribute statistically to assessments of estuarine condition and trends, they provide information that assists in interpreting spatial data. High-frequency water clarity data are also important to understanding seagrass production (Zimmerman et al. 1994) and survival (Moore et al. 1997) because of the natural pulsing of turbidity in estuaries. Zimmerman found that periodic high-turbidity episodes caused by meteorological and tidal forcing events would persist for 1 to 10 days. This duration is short enough that even weekly monitoring at the trend stations could miss them. Similarly, diel changes in dissolved oxygen (D'Avanzo and Kremer 1994) and blooms of chlorophyll (Li and Smayda 2001) all can be missed by higher frequency monitoring.

The bulk of the SOP provides specific information about the methods used to generate a probability-based sampling design compatible with the EPA approach for the National Coastal Assessment. These designs have already been completed and need not be revisited unless the survey design is changed or a new set of random sampling points is needed. GIS coverages and sampling station locations are included as electronic files for future use. Thus, a large portion of the information contained herein serves to document the method and the park-specific details of how it has already been applied. The remainder of the SOP provides guidance on how to apply the spatial design for field surveys. This includes criteria for selecting or rejecting sites, selecting trend stations, and selecting continuous monitoring stations.

2.1.2 *Probability-Based Spatial Survey Design*

Probability surveys use a statistical approach to provide a cost-effective, scientifically-defensible method to determine the overall condition of estuarine waters within and among the NACP and to provide an estimate of the uncertainty surrounding that estimate of condition. Key to the probability survey design is the site selection process. This process requires a delineation of the estuarine area of interest within each park (the spatial domain) and a method for site selection that includes randomization. The probability sampling approach adopted for the NACP includes spatial balance, and for some parks, a stratification of effort among different estuarine components.

The first step for all parks was to identify a spatial domain for sampling. In no instance was an estuary wholly contained within the boundary of a park unit, and some level of subjectivity was required to delineate the area of interest. The boundaries reported here were determined in consultation with staff of the Vital Signs networks and the resource management programs at individual parks to address dominant regional and local interests and monitoring needs. In some cases the entire estuary is included in the spatial sampling domain whereas in others it is not. Because of the very limited relationship between Sagamore Hill National Historic Site and Cold Spring Harbor, no spatial sampling is being recommended for this park. Instead, a single continuous monitoring station will be used during the index period to track water quality conditions.

Two basic sampling designs were implemented for the NACP estuaries to achieve both spatial balance and randomness. The preferred method was to treat each park's estuary as a continuous resource. The spatial domain is overlain by a grid of tessellated hexagons, and one random site is selected for monitoring within each hexagon; each point in the estuary has an equal probability of being sampled. This approach is known as a randomized-tessellation stratified (RTS) design, and was developed to permit control of the spatial dispersion of the samples with explicit recognition of the continuous nature of ecological resources (Stevens 1997). Two parks, Cape Cod National Seashore and Acadia National Park, consisted of more than one estuarine system and/or contained unique subsystems which required a stratification of effort. In these cases, a multiple-density randomized tessellation stratified (MD-RTS) design allowed sampling effort to be distributed evenly among the different estuarine systems/strata.

A base grid of 30 tessellated hexagons is developed for the spatial domain of each park. Hexagons are numbered from 1 to 30 in rows running from west to east, starting with the southern most row and working north. Each year, a new randomly generated point is

selected for monitoring within the estuarine domain of each hexagon. Consideration is made for points that cannot be monitored for logistical reasons, and for hexagons slated for trend monitoring.

Sampling schemes were created using Workstation ArcInfo 8.3 using a custom script (PROCNPS.AML) developed at the U.S. EPA Atlantic Ecology Division, Environmental Effects Research Laboratory in Narragansett, RI. This same script is used for the EPA National Coastal Assessment (current contacts are Walter Galloway galloway.walt@epa.gov and Gerald Pesch pesch.gerald@epa.gov). This script requires seed values for the hexagon cell size, minimum and maximum x & y tics for the tessellation scheme, minimum and maximum x & y bounding coordinates for random point generation, and a number of random points to generate. The values used to generate the RTS designs are provided for each park or park stratum. Thirty randomly selected points have been generated for each of the hexagons of each park or park stratum (Microsoft Excel workbook Appendix 1). Each year, a random sampling location will be selected within each hexagon by progressing through this list in sequence. Once all 30 points within a hexagon have been exhausted, new random points must be generated.

2.1.3 *Trend Stations*

Trend-station monitoring addresses inter-annual change more effectively than can be achieved with annual random sampling alone. Twenty percent of the hexagons in each park (or stratum) are selected as trend stations. To the extent possible, these are distributed systematically throughout the spatial domain. Trend stations are initially positioned randomly within their respective hexagons. Because there is an equal probability that any point within the estuarine domain of the hexagon may be selected, data from trend stations also contribute to the assessment of overall estuarine condition each year (i.e., even though they are not randomly repositioned from year to year). In some cases, NACP parks with existing monitoring programs are able to use their existing permanent monitoring stations for Vital Signs trend stations. There are several conditions that must be met in order for these substitutions to be allowed. First, the sampling location must have been selected to be "representative" of the area from which it was selected, and must be considered representative of the hexagon within which it falls. As such, its location should not have been selected to target a specific area of concern (such as a waste or storm-water discharge pipe or particular basin) unless that concern is representative of the entire hexagon. Also, trend stations must be distributed around the estuarine spatial domain. If existing trend-monitoring stations are clumped within one portion of the estuary, then additional trend stations must be established in other areas. In some cases, more than 20% of the hexagons may already contain suitable trend monitoring stations. Although using all of them does have some effect in weakening the randomization goals of the sampling design, in most circumstances it is not necessary to add randomly-repositioned stations in these. For some of the parks, trend stations have already been identified (see section 2.1.6 below). For the rest of the parks, specific hexagons have been slated for trend monitoring, but selection of the specific point cannot be completed without a reconnaissance trip to assure the suitability of the randomly selected point. Selection or rejection of random sampling points as trend stations shall be conducted in like manner to the probability stations (see section 2.1.4).

2.1.4 *Field methods for the Spatial Survey*

Field crews shall perform a thorough reconnaissance of the survey area well in advance of the index period. They must become familiar with general navigation within the survey area, identify particular hazards to navigation, and attempt to identify stations that may be too shallow for sampling and any other potential problems they might face during the index period. Reconnaissance trips also provide an opportunity to make logistical preparations for fieldwork.

For each park, all spatial and continuous monitoring of water quality vital signs shall occur during a four-week index period. This index period must be scheduled for four continuous weeks during the period from July 1 to August 31 each year. All spatial sampling shall be divided evenly among four weekly survey cruises. For each cruise, all of the trend stations (six) shall be sampled as well as a quarter of the remaining, or annually repositioned, stations (also six). These latter stations should be assigned into the four different cruises to achieve the best spatial balance for each cruise.

The continuous monitoring station must also be visited during each of the weekly cruises. Grab samples for extractive chlorophyll-*a* analysis from this site are used to calibrate the logging chlorophyll-a sensor, and data from the spatial survey YSI sonde and LiCor PAR profiler are used to post-check all the continuous sensors and correct for any sensor drift (see SOP 4, Continuous Water Quality Monitoring with the YSI Sonde).

An initial database of 30 random stations has already been generated for every hexagon of every park (or park stratum). Within each hexagon, stations are serially numbered 1-30 and are provided as geographic coordinates of latitude and longitude in decimal degrees expressed to 5 decimal places. The database of random points is available from the Northeast Coastal and Barrier Network (NCBN) Data Coordinator as a MicroSoft Excel workbook (filename sampling_pt_db.xls). For each hexagon, field crews will use WAAS-enabled GPS to locate the first of these serialized random stations for sampling. If this first site is not accessible, too shallow, too rocky to obtain a sediment sample[1], or otherwise unsafe for sampling, then a notation must be made in the appropriate box on the Station Data Sheet, and the crew should proceed on to the next serialized random station. If it not feasible to sample this next random station, then they should move on to the next; and so on. Once a random station has been either sampled or deemed inappropriate, it is struck from the list of random stations. In future years, field crews will start with the next available random station for that hexagon. When all 30 random stations have been exhausted, a new set must be generated using the methods described in section 2.1.2.

2.1.5 *Continuous Monitoring Stations*

At each park, one station is established for continuous monitoring throughout each index period. These stations are not part of the RTS design and are selected using best

[1] Note that the ability to obtain a sediment sample is necessary only for the monitoring of sediment organic carbon, and so is only relevant to trend stations and probability stations during survey years when sediment monitoring is being conducted.

professional judgment. To the extent possible, their positions should be representative of the overall estuary, while also take into account logistical considerations such as ease of access, interference with public use of the resource, and vulnerability to vandalism or incidental damage.

To reduce interference of sun-shadows on measurements of photosynthetically available radiation (PAR), ideal continuous-monitoring stations will be located on structures that are unobstructed to the South. The water depth (at low tide) must be at least 1.25 m at all times in order to accommodate the PAR sensors. This assumes they are packaged to the YSI 6600EDS, but shallower depths (down to 0.85 m) can be accommodated if necessary. This would require separate mounting of the YSI sonde and the PAR sensors so that the sensor array on the sonde stays at least 0.5 m off the bottom, and the upper PAR sensor stays at least 0.25 m below the water surface.

2.1.6 *Individual Park Designs*

The following sections describe the GIS coverages and methods used generate the spatial designs for each of the North Atlantic Coastal Parks. All derived and original-source GIS coverages used for this protocol are available from the Data Coordinator for the NPS Northeast Coastal and Barrier Network.

2.1.6.1 *Acadia National Park (ACAD)*

Vital Signs monitoring of estuaries at Acadia National Park will be focused on Mount Desert Island (MDI). The park's most significant estuarine resources are on MDI, and this is also where the park faces its greatest threat from watershed development. There are three distinct and separate estuaries on MDI that are associated with park lands and are of interest to ACAD. These are Northeast Creek (NEC), Bass Harbor Marsh (BHM), and Somes Sound (SS). The primary estuaries of concern to both the park and the Northeast Temperate Network (NETN) are BHM and NEC, so the 30 tessellated hexagons have been divided between these strata. Because the estuaries are spatially separated and are of very different sizes, an MD-RTS design is implemented for this park, with 15 hexagons assigned to each stratum.

Somes Sound is a fjord-type estuary and has a characteristic shallow sill at its mouth with the potential of limiting the exchange of deep water. Unlike some fjords, however, this estuary does not stratify so severely that bottom waters become anoxic (Doering and Roman 1994). Nutrient loadings to the sound are low, and *in situ* concentrations of nutrients, chlorophyll-*a,* and dissolved oxygen all indicate a pristine system (Doering and Roman 1994). The need for intensive monitoring in Somes Sound is less pressing than for BHM and NEC, yet the Park and/or network may choose to undertake optional monitoring of this resource. For that purpose, we have identified a spatial stratum for this third estuary, and to it we have assigned an additional 15 hexagons. Should the park (ACAD) or network (NETN) choose to implement a reduced monitoring program in the Somes Sound stratum, we recommend that such an effort start simply with measurements of dissolved oxygen concentration in near-bottom water. This should follow the SOPs for spatial survey sampling; however alternative methods may be implemented (such as Winkler titrations on deep grab samples).

2.1.6.1.1 Somes Sound (SS)

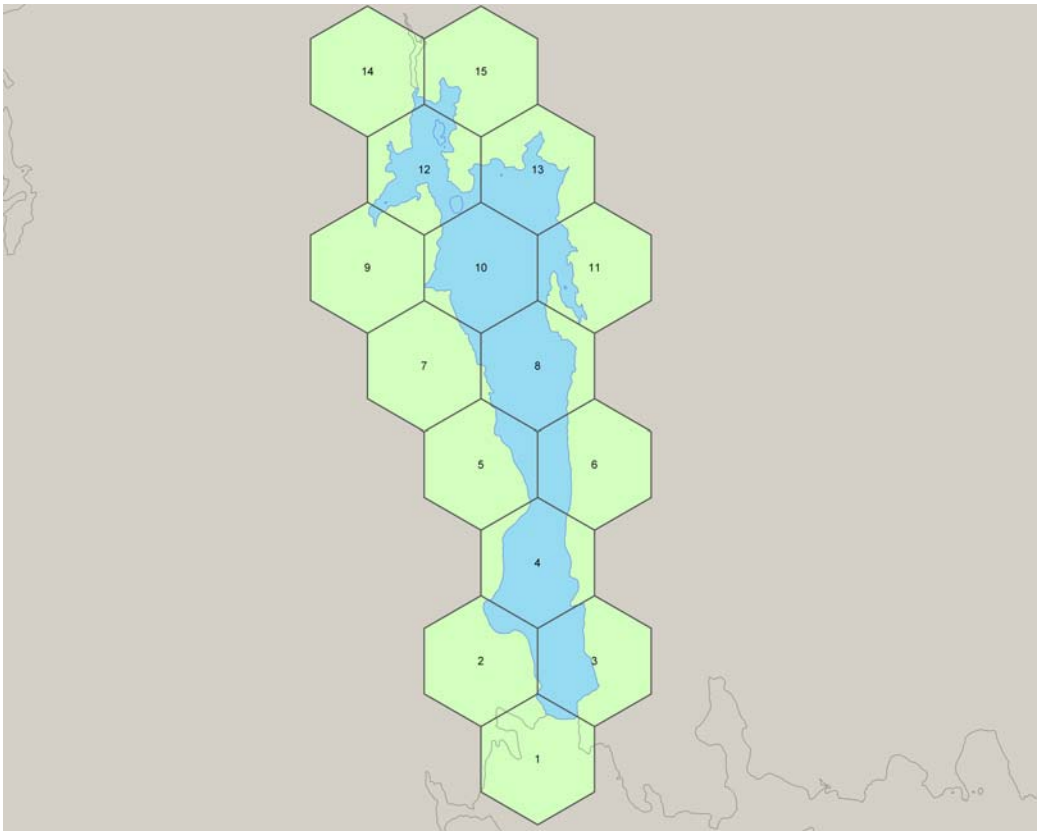

Figure 3. Spatial design for Acadia National Park, Somes Sound Fjord.

The spatial domain for the Somes Sound stratum was created using the following GIS coverages and procedures.

Estuarine boundaries were derived from the coverage "coast" (polygon), 1:24,000 coastline data obtained from Maine Office of Geographic Information Systems (MEGIS, http://apollo.ogis.state.me.us/). Somes Sound is a fjord-like estuary, so the mouth of the estuary was closed off at its shallow sill. This was accomplished using the 30-foot isobath from the file gom15ctr.shp (Digital Bathymetry Contours of the Gulf of Maine) obtained from USGS (http://pubs.usgs.gov/of/of98-801/bathy/data.htm). The spatial domain was closed off at the US Route 102 and 198 bridges using the MEGIS coverage "roads" (line).

Seed values for the PROCNPS.AML algorithm, which was used to generate the tessellated hexagon design and the random sampling stations, were as follows:

- Hex scheme initial tics: x-min = -20000, x-max = +20000, y-min = -20000, y-max = +20000
- Hex Scheme initial cell size: 1422
- Hex scheme initial number of random points: 700,000
- Random Points bounding coordinates: x-min = 552,594, x-max = 556,068, y-min = 4,905,037, y-max = 4,913,359.

Trend stations for this stratum are assigned to hexagon numbers 3, 8, and 12.

2.1.6.1.2 Bass Harbor Marsh Estuary (BHM)

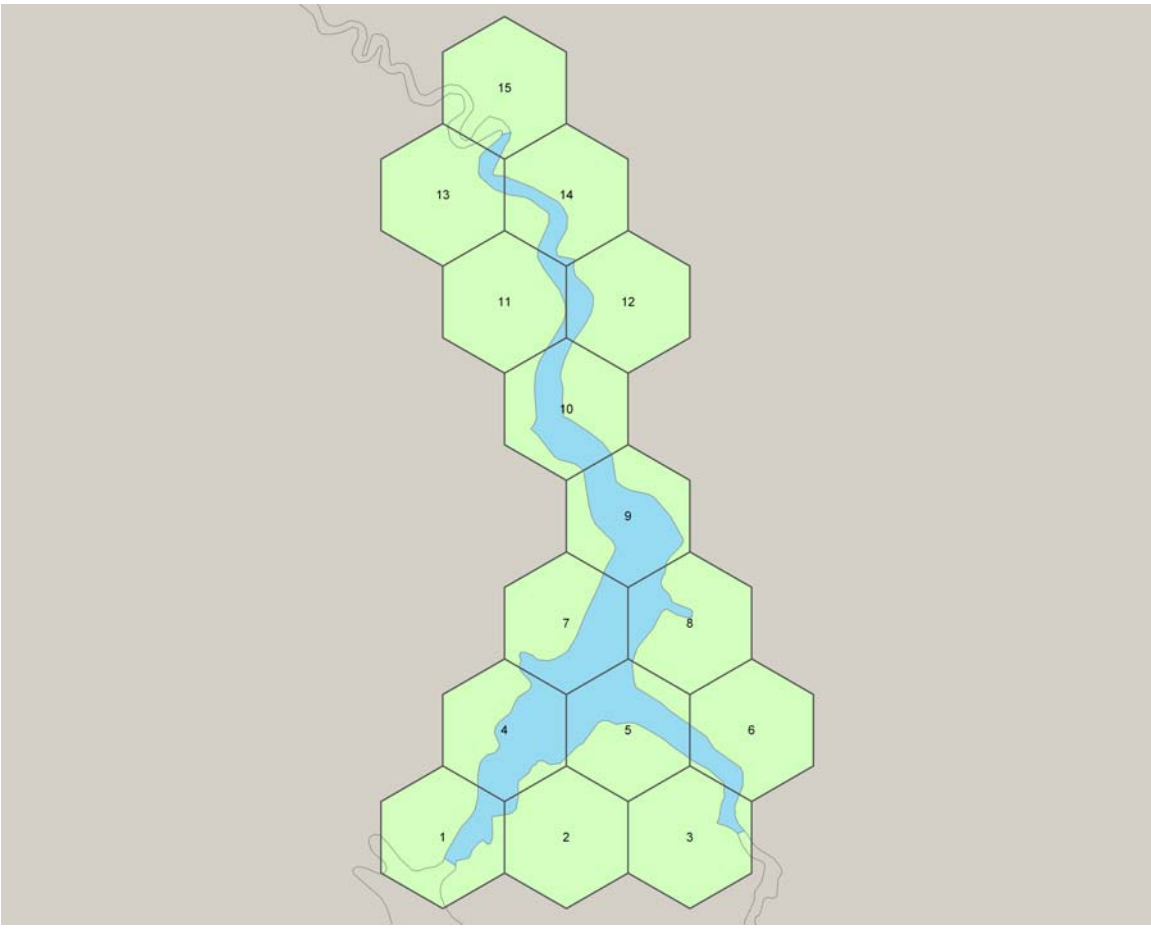

Figure 4. Spatial design for Acadia National Park, Bass Harbor Marsh Estuary.

The spatial domain for the Bass Harbor Marsh stratum was created using the following GIS coverages and procedures. Estuarine boundaries were derived from the coverage "coast" (polygon), 1:24,000 coastline data obtained from MEGIS. The spatial domain was closed off at the two US Route 102 bridges using the MEGIS coverage "roads" (line). The northern extend of the spatial domain was closed off at a point upstream of Buttermilk Brook, defined by the point where the park boundary leaves the estuary toward the east. The GIS park boundary coverage "legbnd2002" (poly), was obtained from ACAD for this purpose.

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -20000, x-max = +20000, y-min = -20000, y-max = +20000
- Hex Scheme initial cell size: 286
- Hex scheme initial number of random points: 900,000
- Random Points bounding coordinates: x-min = 550,940, x-max = 552,970, y-min = 4,889,400, y-max = 4,902,535.

Trend stations for this stratum are assigned to hexagon numbers 4, 9, and 14.

2.1.6.1.3 Northeast Creek Estuary (NEC)

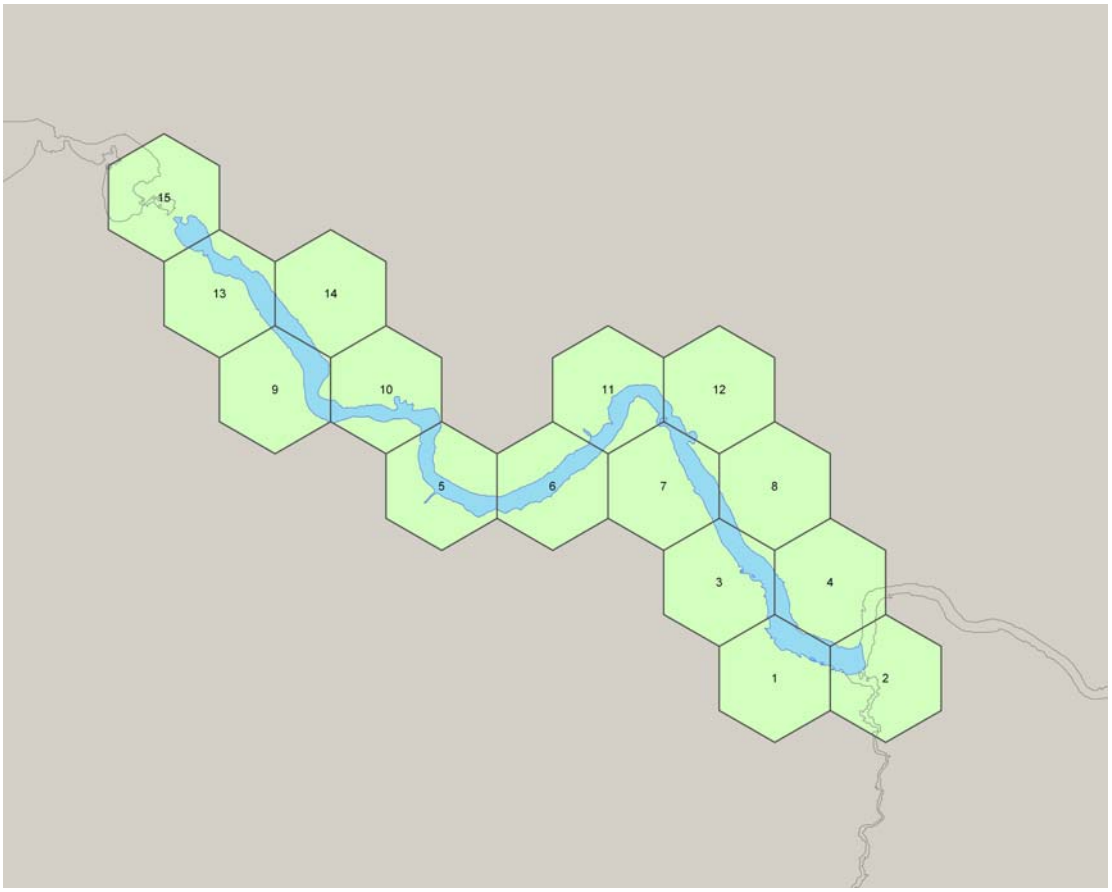

Figure 5. Spatial design for Acadia National Park, Northeast Creek Estuary.

The spatial domain for the Northeast Creek stratum was created using the following GIS coverages and procedures. The shoreline coverage used for the previous two strata was inadequate for delineating this estuary. Instead, the shoreline was based upon the shapefile "roman_shoreline.shp" which was crated as part of a vegetation mapping project by Charles Roman (NPS North Atlantic Coast Cooperative Ecosystem Studies Unit at the University of Rhode Island). The downstream extend of the estuary was cut off at the US Route 3 bridge using the coverage "roads" (line) from MEGIS. The upstream extent was terminated at the confluence of Aunt Betsy's Brook using the MEGIS coverage "hydro" (poly).

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -1000, x-max = +1000, y-min = -1000, y-max = +1000
- Hex Scheme initial cell size: 179
- Hex scheme initial number of random points: 500,000
- Random Points bounding coordinates: x-min = 553,500, x-max = 554,710, y-min = 4,918,500, y-max = 4,919,360.

Trend stations for this stratum are assigned to hexagon numbers 1, 6, and 13.

2.1.6.2 *Assateague Island National Seashore (ASIS)*

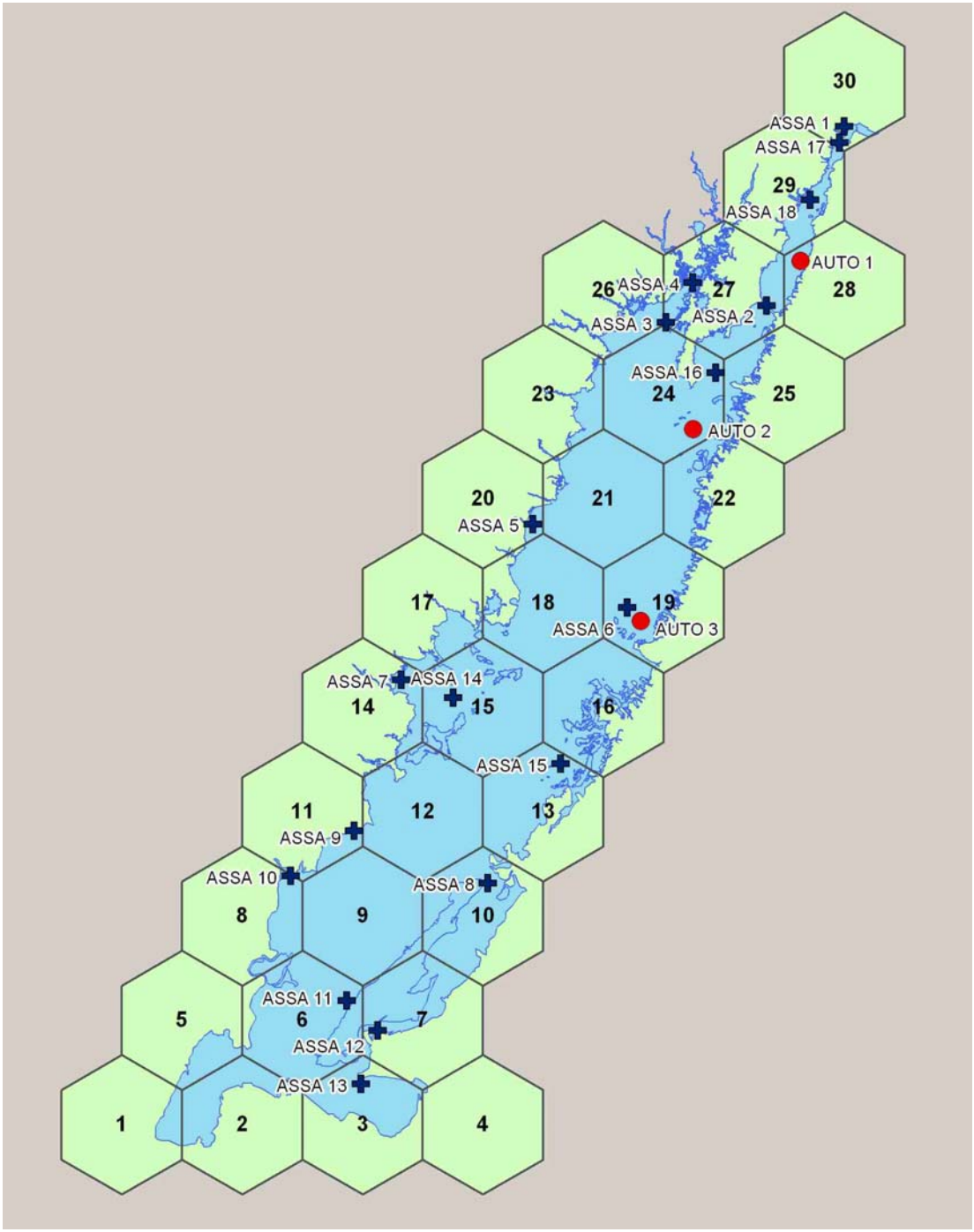

Figure 6. Spatial design for Assateague Island National Seashore.

Assateague is one of two North Atlantic Coastal Parks with a highly developed water quality monitoring program. The management and monitoring of estuarine natural resources at the park is a joint effort among many federal and state partners. The boundary of Assateague Island National Seashore encircles the National Park Service (NPS) holdings, the US Fish and Wildlife Service's Chincoteague National Wildlife Refuge (VA portion of Assateague Island), and Maryland's Assateague State Park.

Further, Sinepuxent Bay and the Maryland portion of Chincoteague Bay are managed under the Maryland Coastal Bays Program (MCBP) as a National Estuary Program (NEP) estuary. Consequently, the Park has monitoring interests throughout the coastal bay, and is not confined in its interests to the area within the park boundary.

Continuous monitoring is conducted at three different stations within the National Seashore boundary, and 16 of the 30 hexagons created for this SOP already have stations where the park monitors an extensive suite of variables (including all the variables required in this protocol). Probability sampling is not currently conducted at ASIS, however, and the Network should facilitate the addition of this component. Of the 18 stations currently monitored, 13 of them in 12 different hexagons qualify as trend stations under the provisions of this SOP (see section 2.1.3 and Table 10). Although this yields more than the desired percentage of hexagons with trend stations (20% target), additional probability samples are not required for any of these hexagons.

Table 10 ASIS water quality monitoring stations eligible and/or selected as trend stations for this NCBN protocol.

ASIS Water Quality Monitoring Station ID	NCBN Hexagon ID	Recommended as a Station & Hexagon for NCBN Trend Analysis?
ASSA 1	30	No- Station is targeted to track problems inside the commercial harbor
ASSA 2	27	Yes
ASSA 3	27	Yes
ASSA 4	27	No- Station is targeted at this largest fresh water input into the bay, which has two point source discharges: a defunct chicken processing plant wastewater treatment facility and the town of Berlin wastewater treatment facility.
ASSA 5	20	Yes
ASSA 6	19	Yes
ASSA 7	14	No- Station was positioned to monitor an area with chronic fecal coliform problems.
ASSA 8	10	Yes
ASSA 9	11	Yes
ASSA 10	8	No- Station is targeted specifically to monitor the mouth of Swans Gut.
ASSA 11	6	Yes
ASSA 12	7	Yes
ASSA 13	3	No- Station was positioned to track effects of aqua culture in this cove - predominantly along the north shore.
ASSA 14	15	Yes
ASSA 15	13	Yes
ASSA 16	24	Yes
ASSA 17	30	Yes
ASSA 18	29	Yes

Estuarine boundaries were derived from the coverage "mdvabays" (polygon), which is a concatenation of a Maryland and a Virginia shoreline coverages created by Luke Cole at the University of Rhode Island. The Maryland shoreline comes from a 2001 shoreline coverage from the Maryland Department of Natural Resources ("DNR_Wetlands" www.MSGIC.state.md.us), and the Virginia portion is from a 2003 edition of the Geographic Data Technology, Inc. U.S. Block Groups coverage produced for the U.S. Department of Commerce, Census Bureau.

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -20000, x-max = +20000, y-min = -20000, y-max = +20000
- Hex Scheme initial cell size: 6100
- Hex scheme initial number of random points: 1,000,000
- Random Points bounding coordinates: x-min = 454,910, x-max = 493,000, y-min = 4,190,100, y-max = 4,244,000.

2.1.6.3 *Boston Harbor Island National Park Area (BOHA)*

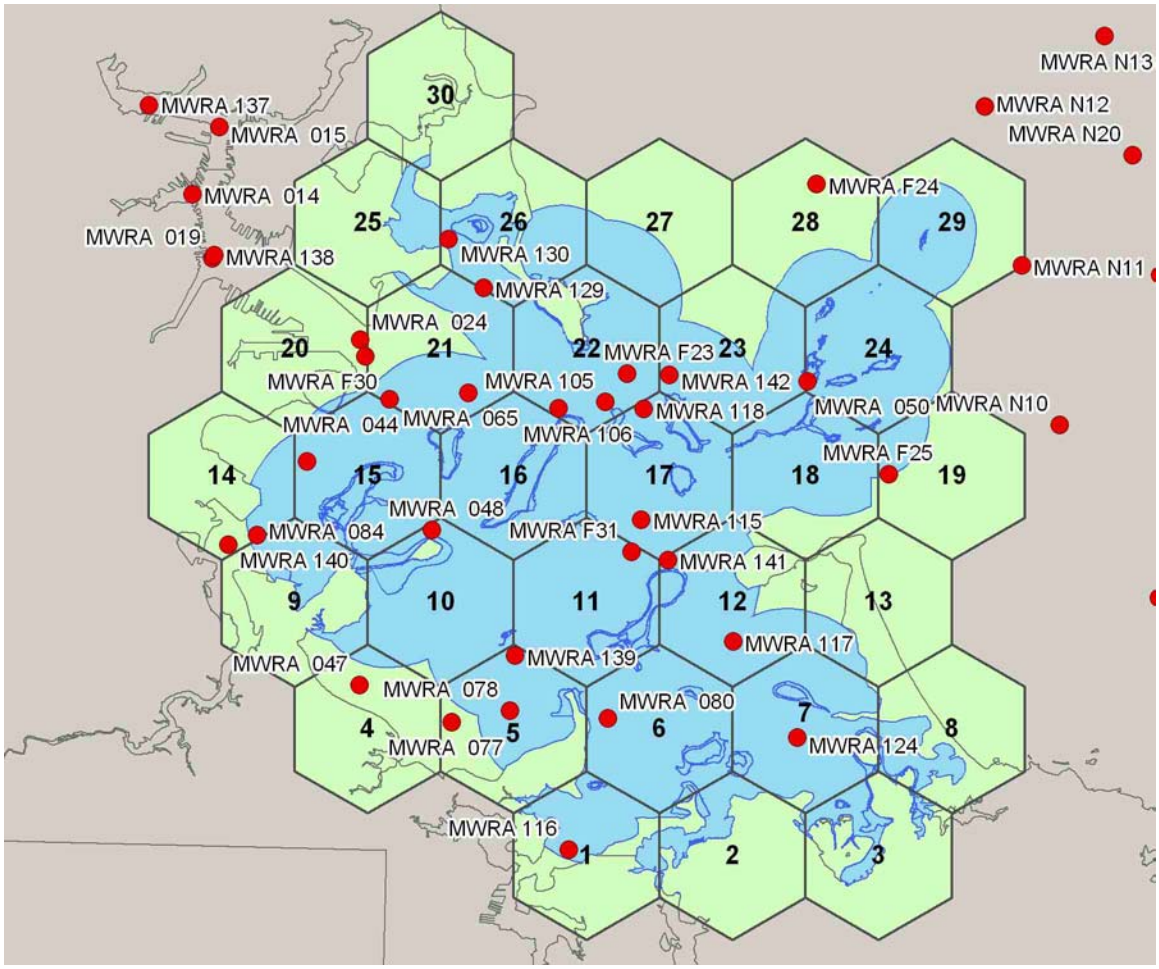

Figure 7. Spatial design for Boston Harbor Island National Park Area.

Although BOHA does not have its own estuarine water quality monitoring program, Boston Harbor has recently been monitored extensively by the Massachusetts Water

Resources Authority (MWRA). MWRA conducts this monitoring as part of their responsibilities surrounding the upgrading of sewage treatment and the relocation of the effluent discharge location (from the harbor to diffusers further out in Massachusetts Bay). With the relocation of the discharge, some of the MWRA stations in the park area have been retired, and the prospects for continued monitoring at other harbor stations is unknown. The present status of MWRA stations and their suitability for Vital Signs monitoring are provided in Table 11.

A study has been proposed to evaluate whether MWRA's monitoring is sufficient for Park and Network management needs and whether it is likely to remain sufficient into the near future (BOHA Scoping Workshop, October 6, 2003). The spatial design provided here is intended to assist in any evaluation efforts by ensuring compatibility with the remaining North Atlantic Coastal Parks. It also provides a design should the Northeast Temperate Network decide to proceed with its own monitoring of BOHA.

The seaward boundary of BOHA extends only to the line of mean low water surrounding the lands owned by the 13-member management partnership (of which NPS is one member). Although the waters of Boston Harbor fall outside the boundary, water quality still plays a direct and significant role in the status and health of natural resources within the park and upon visitor use and enjoyment. On the other hand, suggesting that the park monitor every section of the inner harbor and its coves and tidal creeks would be an overextension of the park's interest and dilute other, more relevant efforts. As a compromise, the spatial domain for monitoring at BOHA was created by establishing a 1.5-km buffer around all the BOHA properties except for the Outer Bay Islands (the Brewsters) where this buffer was reduced to 1 km. Estuarine boundaries were derived from the coverage Boston Harbor Islands Intertidal and Terrestrial Area, "coast" (polygon), obtained from the NPS. The buffered area was saved as a shapefile and combined with the coastline dataset using the ESRI Geoprocessing Wizard, and all upland and intertidal areas were removed from the sampling area using the Edit functions in ArcMap. The GIS coverage "roads" (line) was obtained from the Massachusetts Office of GIS (http://www.state.ma.us/mgis) and was used to crop the spatial domain at Washington Boulevard and at US Route 1.

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -20000, x-max = +20000, y-min = -20000, y-max = +20000
- Hex Scheme initial cell size: 3000
- Hex scheme initial number of random points: 80,000

Random Points bounding coordinates: x-min = 326300, x-max = 351100, y-min = 4676000, y-max = 4695000.

Table 11 Information on MWRA water quality monitoring stations that overlap with the BOHA spatial domain. Variables monitored include (N) "nutrients" includes dissolved and particulate nitrogen and phosphorus, particulate carbon, chlorophyll and total suspended solids; (B) "bacteria" includes *Enterococcus and E. coli*; (P) "physical parameters" includes dissolved oxygen, temperature, salinity, turbidity, transmissivity, and secchi depth); and (C) phytoplankton (chlorophyll) and zooplankton abundance; and (M) primary productivity and respiration.

MWRA Station ID	BOHA Hex- ID	Active Station	Frequency/yr	Variables	Recommended as a Station & Hexagon for NCBN Trend Analysis?
038	15	Y	25	N/B/P	Yes- Station was chosen to track CSO/stormwater bacteria and nutrient effects.
044	21	Y	20	B/P	Yes- Station was chosen to track CSO/stormwater bacteria and nutrient effects.
048	10	Y	20	B/P	No – Station was originally chosen to identify the impacts of a CSO on nearby water quality.
050	24	N	n/a	n/a	No – No longer monitored.
065	21	Y	20	B/P	Yes – Station was originally chosen as representative of the North West Harbor region.
078	05	N	n/a	n/a	No – No longer monitored.
080	06	N	n/a	n/a	n/a - Former treatment plant effluent impact monitoring
084	14	Y	20	B/P	Yes - Neponset River/CSO monitoring
105	22	N	n/a	n/a	No – No longer monitored.
106	22	Y	25	N/B/P	No - Chosen to track nutrient/bacteria conditions off of Deer Island, and changes after completion of various milestones of Boston Harbor Project (BHP)
115	17	N	n/a	n/a	No – No longer monitored.
116	1	N	n/a	n/a	No – No longer monitored.
117	12	N	n/a	n/a	No – No longer monitored.
118	17	N	n/a	n/a	No – No longer monitored.
124	07	Y	25	N/B/P	Yes - Chosen to track nutrient/bacteria conditions in Hingham Bay, site of eelgrass beds, and to track changes after various stages of BHP.
129	26	N	n/a	n/a	No – No longer monitored.
130	26	Y	25	N/B/P	Yes - Chosen to track nutrient/bacteria conditions in Winthrop Bay, site of eelgrass beds, especially after inter-island transfer
139	05	Y	25	N/B/P	Yes - Chosen to track nutrient/bacteria conditions off of Nut Island
141	12	Y	25	N/B/P	Yes - Chosen to track nutrient/bacteria conditions
142	24	Y	25	N/B/P	No - Chosen to track nutrient/bacteria conditions off of Deer Island, and changes after completion of various milestones of Boston Harbor Project (BHP)
F23	22	Y	6	N/P/C/ M	Yes - Originally chosen to help characterize the export of nutrients from Boston Harbor into Massachusetts Bay.
F25	19	Y	6	N/P/C	Yes - Originally chosen to help characterize coastal conditions between Harbor and site of future outfall.
F31	11	Y	6	N/P/C	Yes - Originally chosen to help characterize general water quality in outer Harbor.

2.1.6.4 *Cape Cod National Seashore (CACO)*

Much of the estuarine habitat at CACO is associated with two large coastal lagoon systems, Pleasant Bay (PB) and Nauset Harbor/Marsh (NH). The former has an open-water area approximately four times greater than that of the latter, and so an MD-RTS design is required to distribute sampling effort evenly between them. Each of these lagoons is fringed by small, deep, glacial kettle-hole salt ponds that flush with seawater from the lagoons. Because of their unique nature, these have been separated into a third stratum for separate consideration. The kettle-holes are generally too small to require spatial sampling within any one of them. Instead, they are each treated as discrete spatial elements within the kettle-hole stratum. The Pleasant Bay Resource Management Alliance already conducts enough monitoring in the kettle-hole ponds of PB to meet the monitoring and management needs of the NCBN. Conversely, the kettle-hole ponds flushing into NH will require NCBN monitoring. Salt Pond, a 12 m deep kettle-hole that flushes into NH, falls within the National Seashore boundary and should be equipped with a continuous monitoring station during the summer index period. Mill Pond is not as deep as Salt Pond and consequently not as vulnerable to stratification. Also, it falls outside the seashore boundary. Monitoring that is consistent with both the spatial survey approach (single probability station) as well as those of the Pleasant Bay Resource Management Alliance should be adopted for Mill Pond.

Thirty tessellated hexagons are divided evenly between the NH and PB strata. Spatial domains for each of the strata were created using the following GIS coverages and procedures. Estuarine boundaries are derived from the shapefile “shoreline01” (line), coastline data obtained from NCBN. Salt Pond and Mill Pond kettle-holes were cropped from the NH spatial domain using 1:5,000 Elevation data obtained from Massachusetts Office of GIS to select break lines. Kettle-hole ponds were cropped from the PB stratum by hand so that associated shallow channels were also removed from the PB stratum.

2.1.6.4.1 Nauset Harbor (NH)

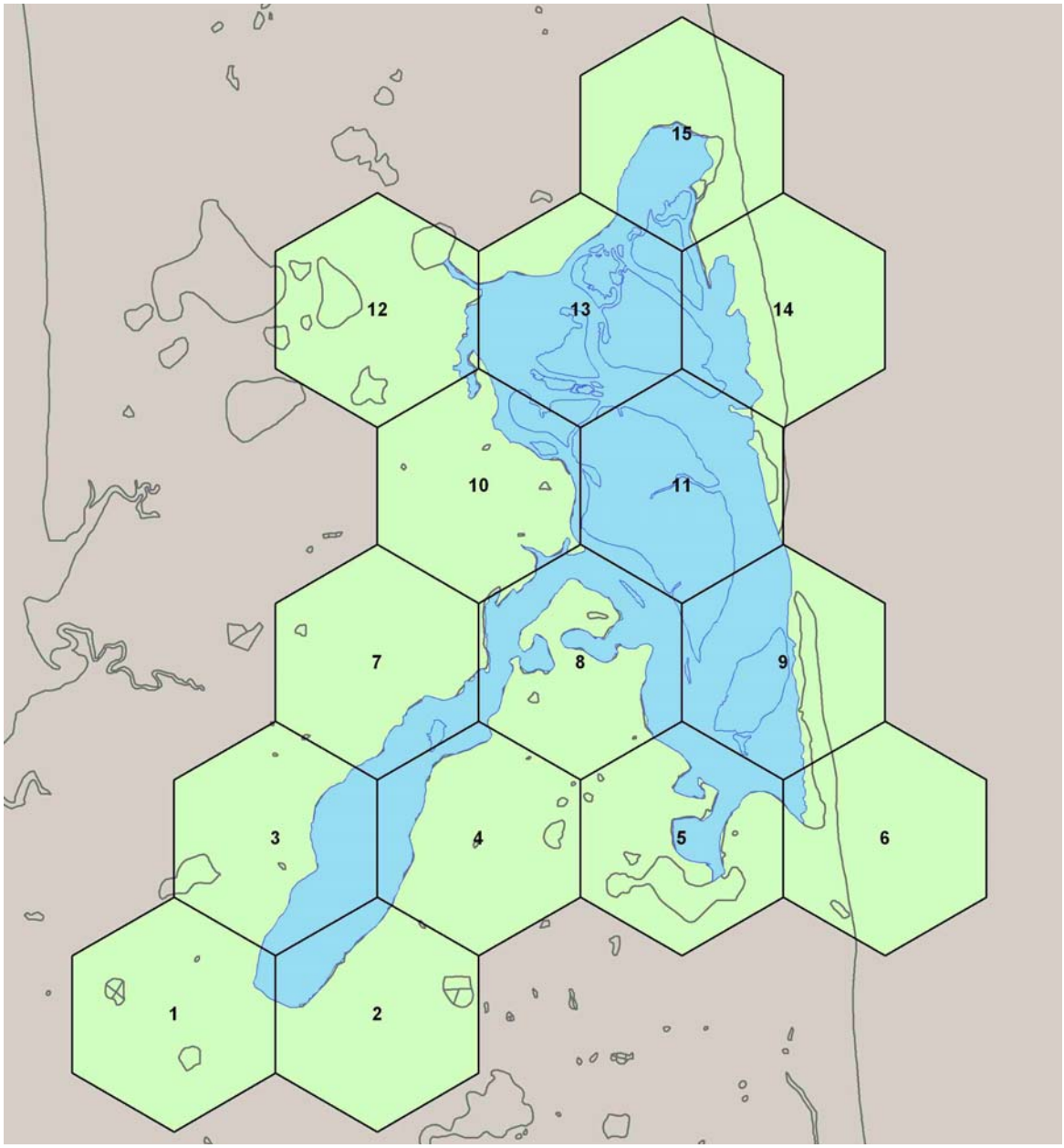

Figure 8. Spatial design for Cape Cod National Seashore, Nauset Harbor/Marsh.

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -20000, x-max = +20000, y-min = -20000, y-max = +20000
- Hex Scheme initial cell size: 1440
- Hex scheme initial number of random points: 900,000
- Random Points bounding coordinates: x-min = 417,678, x-max = 422,039, y-min = 4,626,320, y-max = 4,633,032.

Trend stations for this stratum are assigned to hexagon numbers 3, 8, and 13.

2.1.6.4.2 Pleasant Bay (PB)

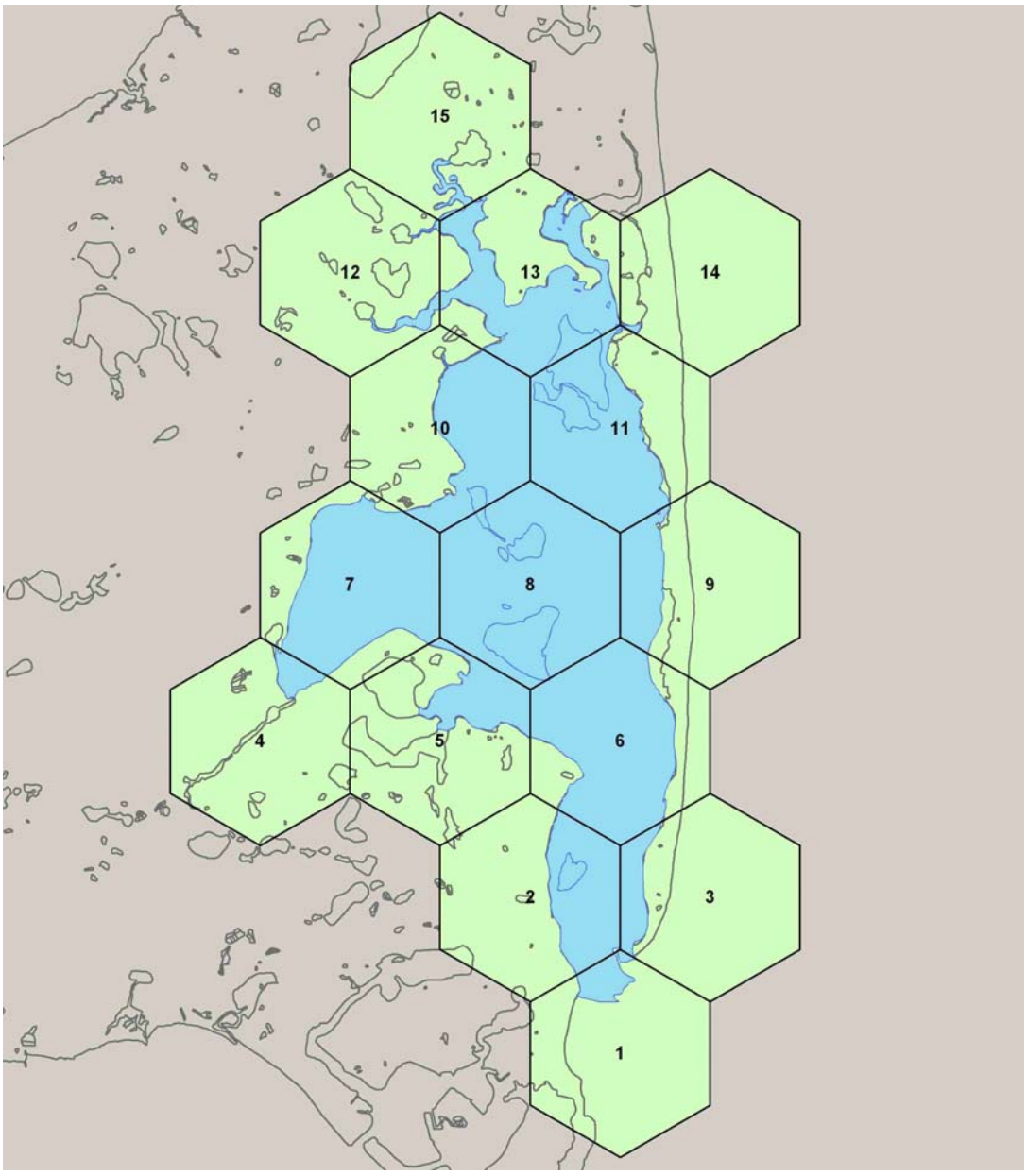

Figure 9. Spatial design for Cape Cod National Seashore, Pleasant Bay.

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -20000, x-max = +20000, y-min = -20000, y-max = +20000
- Hex Scheme initial cell size: 2475
- Hex scheme initial number of random points: 900,000
- Random Points bounding coordinates: x-min = 416,503, x-max = 423,153, y-min = 4,613,887, y-max = 4,626,259.

Trend stations for this stratum are assigned to hexagon numbers 3, 7, and 11.

2.1.6.5 *Colonial National Historic Park (COLO)*

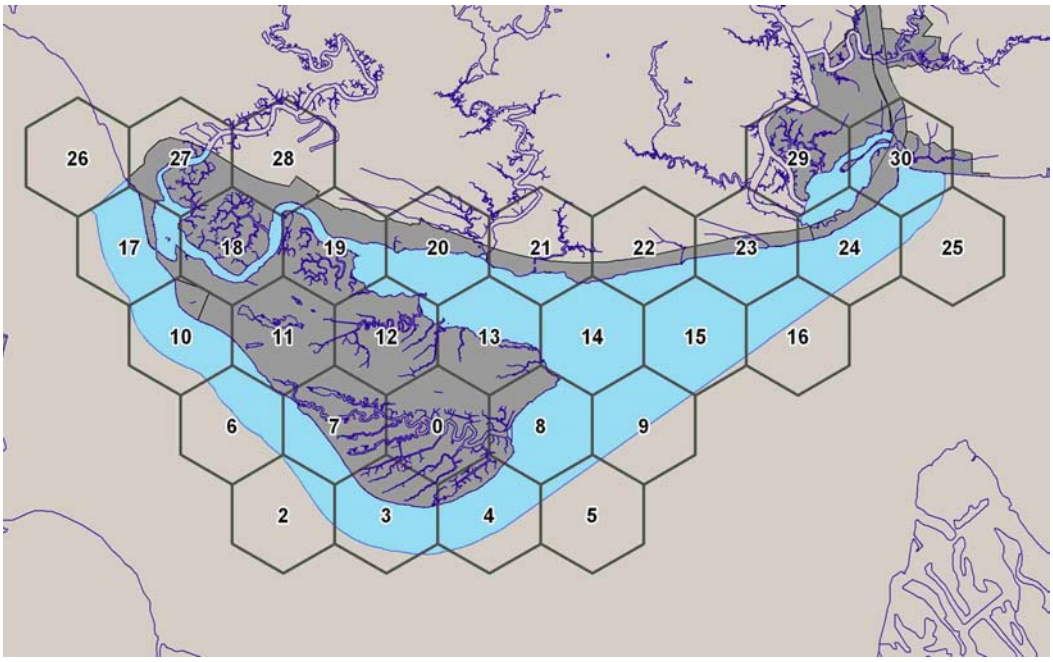

Figure 10. Spatial design for Colonial National Historical Park, Jamestown Island District.

Colonial National Historical Park (COLO) consists of two significant land holdings, the Yorktown and Jamestown Units, connected by a narrow traffic corridor, the Colonial Parkway. Although the Yorktown Unit and Parkway both abut the lower York River and cross over a number of tidal creek systems, the predominant estuarine resources at COLO surround the Jamestown unit. The estuarine habitat surrounding Jamestown is dominated by the lower James River on one side, and by Sandy Bay, Back River, and The Thorofare on the other. While Jamestown Island is itself undeveloped, the Powhatan Creek watershed, which drains into Sandy Bay and The Thorofare, is under tremendous development pressure. Consequently, estuarine monitoring at COLO is focused on this area surrounding Jamestown Island.

The park boundary was used to terminate the spatial domain at Powhatan Creek. Offshore of Jamestown Island, a 500m buffer from the shoreline was used to delineate the spatial domain. A second 500m buffer was created from the shoreline at the mouth of College Creek, and a single tangent was created south of these buffers to join them.

The RTS design for COLO was created by the U.S. EPA Atlantic Ecology Division, Environmental Effects Research Laboratory in Narragansett, RI. The spatial domain was created using the shoreline coverage "hydrology ln" (line) and park boundary coverage "colo boundary" (shape) obtained directly from COLO. The original hex scheme tic marks and cell size are unavailable, but the PROCNPS.AML script was modified to generate additional random sampling points for the original hex scheme as follows.

- Hex scheme initial number of random points: 500,000
- Random Points bounding coordinates: x-min = 339900, x-max = 351300, y-min = 4115300, y-max = 4122600.

Trend stations for this park are assigned to hexagon numbers 1, 10, 15, 18, 20, & 29.

2.1.6.6 *Fire Island National Seashore (FIIS)*

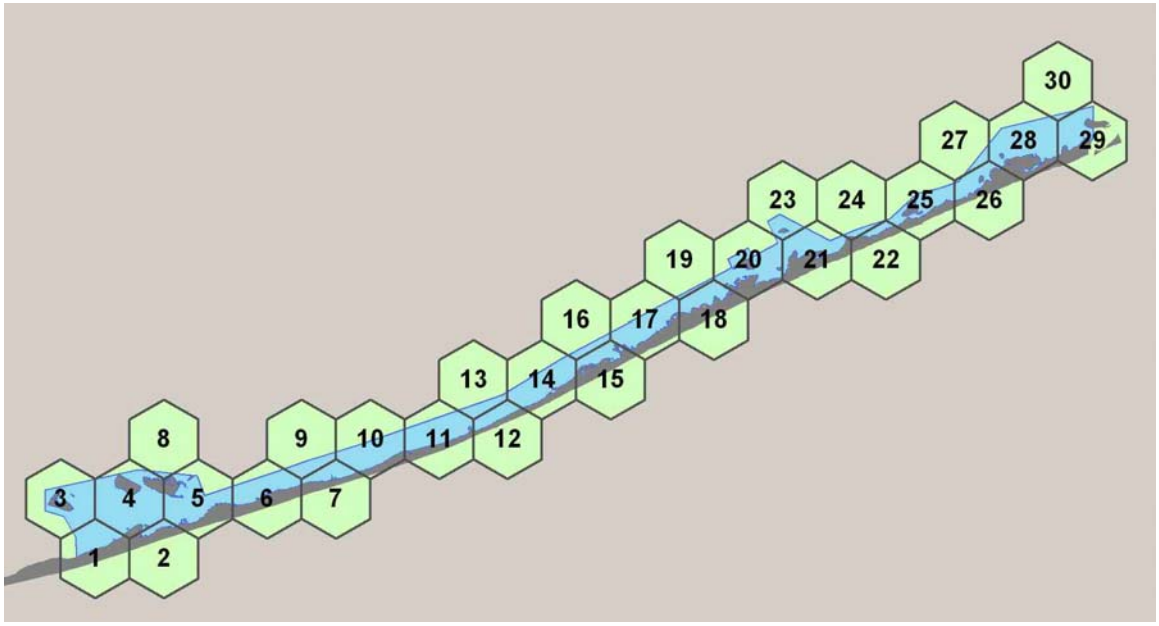

Figure 11. Spatial design for Fire Island National Seashore.

Fire Island National Seashore (FIIS) is a 7,810-ha park on the South Shore of Long Island, New York. Approximately 46% of this area is submerged estuarine land in Great South and Moriches Bays. Although FIIS owns only a minority portion of the submerged land in these bays, this area in park ownership is, nonetheless, extensive. Rather than dilute the sampling intensity by extending into the coastal bays at large, we chose to focus NPS monitoring on the area within the seashore boundary. This decision was bolstered by an understanding that the South Shore Estuary Reserve intends to promote and expand water quality monitoring within Great South Bay as a whole.

The RTS design for FIIS was created by the U.S. EPA Atlantic Ecology Division, Environmental Effects Research Laboratory in Narragansett, RI. The spatial domain was created using the following GIS coverages and procedures. Estuarine boundaries were derived from the coverage "park_boundary" (polygon) and the shoreline coverage "poly_island" (polygon), both obtained directly from FIIS. The original hex scheme tic marks and cell size are unavailable, but the PROCNPS.AML script was modified to generate additional random sampling points for the original hex scheme as follows:

- Hex scheme initial number of random points: 500,000
- Random Points bounding coordinates: x-min = 339900, x-max = 351300, y-min = 4115300, y-max = 4122600.

Trend stations for this park are assigned to hexagon numbers 5, 10, 15, 20, 25, & 30.

2.1.6.7 *Gateway National Recreation Area (GATE)*

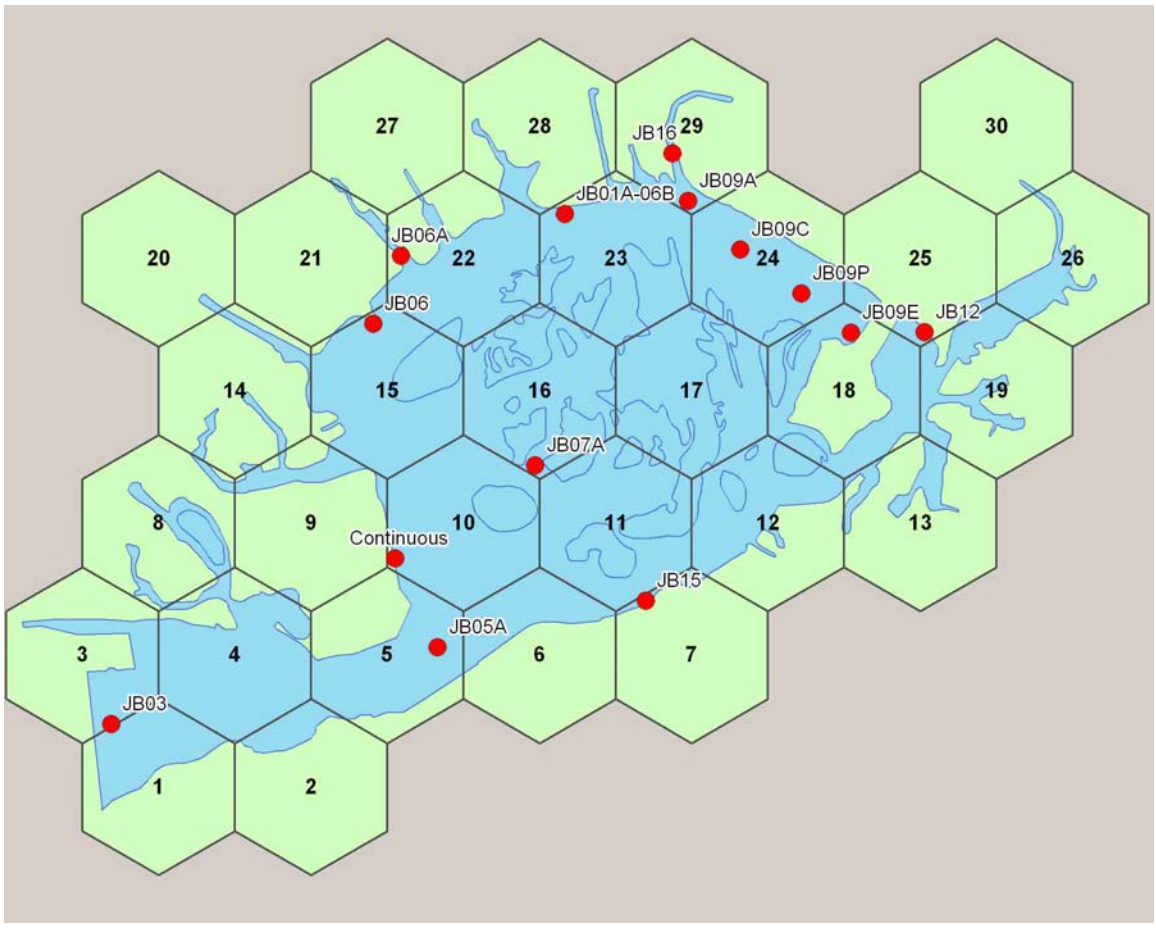

Figure 12. Spatial design for Gateway National Recreation Area.

Gateway National Recreation Area (GATE) consists of 10,783 hectares of coastal uplands, freshwater ponds, marshes, bays, beaches, and mudflats. Established in 1972, it is divided into several geographically separate units. These are the Jamaica Bay/Breezy Point units, the Sandy Hook unit, and the Staten Island unit. In New York, all submerged lands within one quarter mile of any Park-owned shoreline are explicitly included in the enabling legislation for GATE. In New Jersey, however, the state claims ownership of the bottom, and consequently GATE does not regulate submerged lands at the Sandy Hook Unit. Thus, estuarine resources at GATE are dominated by Jamaica Bay.

Gateway is one of two North Atlantic Coastal Parks with a highly developed water quality monitoring program. A spatial monitoring program has been in effect at Gateway since 1977, and 13 permanent trend stations are currently part of the Jamaica Bay water quality sampling program. These 13 stations fall within 11 of the 30 hexagons created for this SOP, and monitoring by the park includes most of the variables specified by this protocol. Probability sampling is not currently conducted at GATE and the Network will need to facilitate the addition of this component to the ongoing water quality monitoring program. Of the 13 stations currently monitored, ten of them, falling within nine different hexagons, qualify as trend stations under the provisions of this SOP (see section 2.1.3 and Table 12). Although this yields more than the desired percentage of hexagons

with trend stations (20% target), additional probability monitoring is not required for any of these hexagons.

Table 12 GATE water quality monitoring stations eligible and/or selected as trend stations for this NCBN protocol.

GATE Water Quality Monitoring Station ID	NCBN Hexagon ID	Recommended as a Station & Hexagon for NCBN Trend Analysis?
JB 03	3	Yes
JB 05A	5	Yes
JB 15	7	Yes (although this is a targeted site, the hex element is small enough that the station is still representative
JB 07A	16	Yes
JB 06	15	Yes
JB 06A	22	No- Station was positioned to monitor a stormwater discharge.
JB 06B	23	Yes
JB 16	29	No- Station was positioned to monitor a stormwater discharge.
JB 09A	29	Yes
JB 09C	24	Yes
JB 09P	24	Yes
JB 09E	18	Yes
JB 12	25	No- Station was positioned to monitor a stormwater discharge.

The spatial domain for GATE was first created by the U.S. EPA Atlantic Ecology Division, Environmental Effects Research Laboratory in Narragansett, RI. Following the 2003 feasibility study, and in consultation with the park, the spatial domain has since been modified to include several previously excluded tributaries. This required a revision of the RTS design as well.

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -19400, x-max = +20000, y-min = -19500, y-max = +20000
- Hex Scheme initial cell size: 2550
- Hex scheme initial number of random points: 100,000
- Random Points bounding coordinates: x-min = 584730, x-max = 611470, y-min = 4487300, y-max = 4506020.

2.1.6.8 *George Washington Birthplace National Monument (GEWA)*

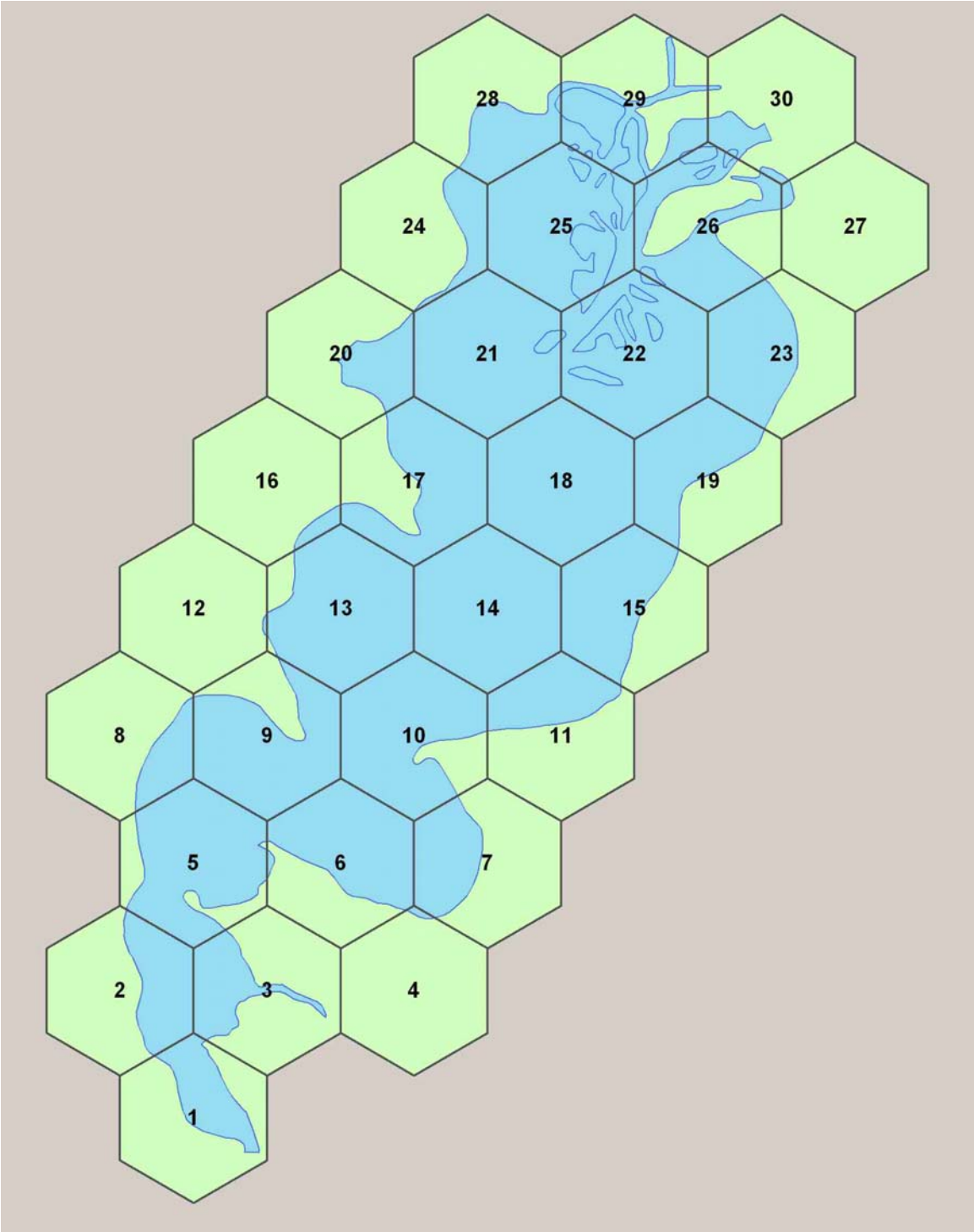

Figure 13. Spatial design for George Washington Birthplace National Monument.

George Washington Birthplace National Monument (GEWA) is a small, 223-ha unit of the National Park Service located along the tidal reaches of the Potomac River in Westmoreland County, Virginia. The Potomac shoreline delineates the northern boundary of the monument, and three small sub-basins drain into the Potomac at this

location. The largest of these sub-basins, and the most significant estuarine resource at GEWA, is Popes Creek. Because of the monitoring efforts of the Chesapeake Bay Program, adequate data exist to evaluate trends in water quality in the Potomac River itself, and NPS efforts should be focused on Popes Creek.

The RTS design for the GEWA was created using the following GIS coverages and procedures. Estuarine boundaries were derived from the coverage "gewastrm" (polygon), George Washington Birthplace NM Hydrography, 1:24,000 hydrology data developed by NCSU. The Maryland-Virginia border was used to close the spatial domain, using the coverage "esri_states" obtained from Environmental Systems Research Institute, Inc.

Seed values for the PROCNPS.AML algorithm were as follows:

- Hex scheme initial tics: x-min = -20000, x-max = +20000, y-min = -20000, y-max = +20000
- Hex Scheme initial cell size: 336
- Hex scheme initial number of random points: 100,000
- Random Points bounding coordinates: x-min = 330000, x-max = 334000, y-min = 4226000, y-max = 4230000.

Trend stations for this park have been assigned to hexagon numbers 1, 6, 13, 18, 23, & 25.

2.1.7 *References*

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2.2 SOP 2 – Field Season Logistics

2.2.1 *Field Crew Training*

The Standard Operating Procedures included in this document are intended to be a comprehensive description of the methods associated with Vital Signs monitoring in North Atlantic Coastal Park (NACP) estuaries. They are not, however, intended to be a training manual for field or laboratory personnel. Whether staffed by NPS employees or independent contractors, Vital Signs personnel will need additional training and support in order to successfully and safely complete the work described herein. Safety, in particular, must always be the highest priority. All NPS employees doing field or laboratory work for this protocol should be trained in cardio-pulmonary resuscitation (CPR) and basic first aid. Employees working in laboratory settings must receive safety training to do so even if their immediate work tasks involve no hazardous substances (dry ice is a safety concern for both lab and field personnel however). Likewise, all US Department of Interior employees who operate motorboats must receive training and safety certification to do so (DOI Motorboat Operator Certification Course). A basic assumption of this program is that all DOI and NPS safety guidelines will be followed while completing the work. Regardless of the methods provided in this protocol and regardless of existing DOI and NPS safety guidance (or lack thereof), it is the job of each employee to work safely. Should the methods in this document contradict your own sense of safety, then it is your obligation to discuss this concern with Network supervisors.

In addition to the safety training, field crews will need general training on protocol implementation for conducting all water column, sediment, and seagrass monitoring. Additionally, personnel will require detailed training on calibration, programming and use of the YSI and LiCor instruments. It is outside the scope of this protocol to review all of the information and methods that are required to operate the various YSI and LiCor instruments and to make effective and accurate measurements. It is imperative that both field and laboratory personnel familiarize themselves with the entire YSI 6-Series Environmental Monitoring Systems Operations Manual, the Multiparameter Display System Operations Manual, and the Technical Notes and Technical Documents available from YSI at their web site. YSI, Inc. offers coursed on instrument calibration and maintenance. We strongly advise that all NACP personnel involved in instrument calibration receive annual training, either directly from YSI or from a NACP representative who has recently completed YSI training.

2.2.2 *Field Season Preparation*

The ability to successfully complete the annual monitoring will hinge upon thorough preparation in advance of the field season. The most important preparation is to ensure that the program is adequately staffed to accomplish the fieldwork. There are three different models that were used during pilot testing of water-column monitoring procedures (spatial surveys and continuous monitoring at logging stations), and each of these will require its own logistical preparations.

4) Monitoring Conducted by the Individual Park –

GATE and ASIS have well-established monitoring programs for estuarine water quality. For these parks, collaboration will likely be mutually beneficial to both the Northeast Coastal and Barrier Network (NCBN) and the Park. The nature of any cooperative agreement must be negotiated, but a likely model is for the Network to provide funding and/or seasonal staff to offset the additional cost of the Network monitoring protocol.

5) Monitoring Conducted by an Academic Institution –
For some of the parks, it may be advantageous for the Network to contract out the monitoring work to an academic institution. COLO and GEWA are examples where this model would work very well. The Virginia Institute of Marine Science (VIMS)/Chesapeake Bay National Estuarine Research Reserve in Virginia is preeminent with its expertise in estuarine monitoring and their investigators already conduct very similar monitoring with the same or similar equipment in the nearby vicinity of COLO and GEWA. Cooperative agreements for such work will need to be negotiated annually.

6) Monitoring Conducted by the Network –
For parks where neither of the above options is logistically or economically feasible, monitoring work will fall to the Network.

In addition to staffing and establishing contracts for services, many of the SOPs in this protocol require preparatory steps that must be completed well in advance of the field season. Field and laboratory equipment used for each SOP must be thoroughly inspected, tested, and repaired or replaced as necessary; expendable supplies must be stocked; and field crews must receive safety and protocol training. For all three of the logistical models, the Network must play an active role in methods training for field personnel to ensure the protocol is implemented properly and to assure data consistency and quality. Notable preparations that may require additional lead time are listed in Table 13.

Experience gained during the feasibility test phase of this protocol suggests that a field crew of two people can manage the water quality monitoring at two parks, and three people if boat logistics are complicated. Four people are required for the 3 -4 days associated with seagrass condition monitoring (for two sites). This number assumes the site is already set up and intact. Initial set-up will require six people over two days for each monitoring site.

Table 13. Preparations for index-period monitoring requiring exceptional lead time and/or advanced planning.

SOP #	SOP Topic	Advance Preparations
3	Continuous logging Station	- If installation of continuous monitoring station is to be permanent, obtain applicable PAToN permit from US Coast Guard. - If installation is to be temporary (<6 months) then make appropriate notifications to US Coast Guard. - Construct and install support structure and mounting brackets in advance of index period.
4&5	YSI-water quality sonde	- Obtain calibration training from YSI, Inc. - If purchasing new equipment, note that the YSI for SOP 4 is factory customized and may require additional lead time to fill the order.
4&6	LiCor- PAR equipment	- Obtain factory re-calibration of PAR sensors. Calibrate biennially for sensors used only in spatial monitoring and annually for sensors used in continuous monitoring.
7	Discrete chlorophyll-*a*	- Identify an analytical laboratory to analyze discrete grab samples for chlorophyll-*a*. Enter into a contract agreement that includes specific on all required data quality assurance measures.
8	Sediment TOC	- Identify laboratory to analyze sediment samples for TOC. Enter into contract agreement that includes specifics on all required data quality assurance measures.
3-8, 10	Motorboat Operations	- -Train and certify boat operators with a DOI approved Motorboat Operator Certification Course (valid for 5 years).
10	Seagrass Condition	- Identify monitoring site and establish monitoring transects - Identify laboratory to analyze sediment samples.

Schedule of training and field activities

June: Safety training
Motorboat training
General training for water quality monitoring
YSI and LiCor instrument training
Installation of a support structure to hold a continuous water quality sonde
Reconnaissance and familiarization with the field setting

July: Seagrass condition training
Seagrass condition monitoring
Initiation of continuous monitoring at the logging station
Initiation of weekly spatial water quality cruises

August: Continuation of weekly survey cruises
Continuation of continuous monitoring at the logging station
Completion of index period monitoring

Late August – Early September
Removal of temporary logging station fixtures
Organize and review all data sheets and digital data files
Clean, service, and store all equipment

2.3 SOP 3 – Preparation of the Logging Station

2.3.1 *Introduction*

This SOP describes methods for permitting, installing, and maintaining stations for continuous monitoring of water quality using the YSI 6600EDS multi-parameter sonde. North Atlantic Coastal Parks (NACP) are quite different, and this SOP allows for a variety of approaches to constructing continuous monitoring stations. The exact architecture of the logging stations is flexible to accommodate variation in geographic setting, vulnerability to inadvertent damage or vandalism, and logistical considerations. Consequently, some portions of this SOP provide general guidance while other portions firmly prescribe standard methods necessary to achieve performance standards and data compatibility. This distinction is emphasized where applicable in the SOP.

2.3.2 *Design and Permitting of Support Structure*

The EDS YSI sonde must be mounted on a pier, piling, or other support structure during a 4-week late summer sampling period. Infrastructure requirements vary for each park, and not all parks need new pilings or work platforms for continuous water-quality monitoring. Parks should be surveyed initially for existing structures that could be used to support the monitoring equipment. At GATE, for example, an existing pier provides an ideal mounting structure, and ASIS already has established monitoring platforms. For other parks, temporary pilings that are installed and removed seasonally may be preferable to permanent structures. For example, Great South Bay freezes over many winters, and ice damage to pilings can be severe. Rather than design an installation to withstand the ice, it may be preferable for FIIS to simply install a more modest piling each summer and remove it each fall.

When designing or selecting a support structure upon which the YSI 6600EDS will be mounted, it is important to keep the following design features in mind. Because the 6600EDS used for this protocol is modified to measure underwater PAR (using two LiCor radiometers), it is important that the piling, pier or other support structure not shade the instrumentation during any part of the day. At the latitude of NACP, this is generally achievable by ensuring that the support structure is unobstructed and has no overhang to the south. Examples of structures included in the feasibility test of this protocol include a pier at Gateway National Recreation Area with good southern exposure (Figure 14a), a single 4x4 wooden piling jetted 2 m into the sand at Fire Island National Seashore (Figure 14b), and a more permanent monitoring platform installed at Colonial National Historical Park following the design of the Virginia-Chesapeake National Estuarine Research Reserve (Figure 14c).

Figure 14. Logging stations for the 2003 feasibility test. (a) at NYPD Pier, Floyd Bennett Field, GATE; (b) temporary 4x4 piling jetted in near Bennett Beach, FIIS; (c) logging platform installed in the style of the VA-Chesapeake NERR at COLO.

Permitting requirements depend on whether the logging platform is a new, permanent structure. Title 33 of the Code of Federal Regulations, Subchapter C, Part 66 describes the regulation of Private Aids to Navigation (PAtoN) by the U.S. Coast Guard. The installations shown above for FIIS and COLO, if permanent PAtoNs, would most likely be considered information or regulatory markers (warning day beacons). Importantly, a distinction is made between those "private aids" that are permanently installed, and those that are considered temporary. "Temporary aids" are those that will be on station six months or less. These aids do not require a PAtoN application, but they do require notification to the Coast Guard by letter, fax, or email, for publication in the Local Notice to Mariners. Another advantage of a temporary aid is a reduced burden of maintenance. The locations of permitted PAtoNs are printed on NOAA navigation charts and carry an expectation that the owner keep them maintained throughout the year.

Only the US Coast Guard and the Department of Defense are exempt from the requirements of CFR Title 33 Part 66. The 13th Coast Guard District has produced an information handout that includes applicable edited material from the CFR and helpful instructions explaining the application procedures (Appendix 2). Also appended (Appendix 3) is a copy of the application form (CG-2554). This form is submitted to the District Commander of the applicable USCG district (Table 14). For GEWA, COLO, and ASIS, this is the Fifth District, and for ACAD, BOHA, CACO, SAHI, FIIS, and GATE, this is the First District. In most cases, the location of the permanent water quality monitoring stations will be outside major boat traffic channels; however, fixed structures (such as pilings) present potential hazards to navigation and may require additional review and authorization from the Corps of Engineers, Department of the Army (Code of Federal Regulations; Title 33, Part 66.01-30). This review and authorization would be conducted by the District Engineer of the applicable district . It is recommended that an advance application (CG-2554) be submitted to the USCG District Commander in order to solicit recommendations on the need for Army Corp review and any suggestions for the formal application.

Table 14. Contact information for PAtoN applications.

District	Mailing Address	Current Point of Contact
First	Commander (Oan) 1st Coast Guard District 408 Atlantic Ave. Boston, MA 02110,	Jack McLaughlin PATON Manager 1st Coast Guard District (Oan) 617-223-8358 voice 617-223-8073 fax paton@d1.uscg.mil
Fifth	Commander (Oan) 5th Coast Guard District 431 Crawford Street Portsmouth, VA. 23704	Albert L. Grimes III Waterways Manager (VA&MD) 5th Coast Guard District (Oan) Waterways Management Branch 757-398-6360 voice 757-398-6334 fax AGrimes@lantd5.uscg.mil

2.3.3 *Deployment Tubes and Brackets*

When deploying the monitoring sonde in unattended mode, protection from accidental damage, vandalism, debris, fouling, and animals will not only improve the data quality, but it can also reduce the number of site visits required for servicing. Some sort of mounting tube is recommended for all sites in the NACP. The standard mounting tube recommended by YSI Inc application engineers is a length of schedule 40 PVC pipe drilled with cross holes and painted with ablative marine antifouling paint antifouling paint (Figure 15). A pipe cap is affixed to the top of the pipe, and drilled to accept a length of chain and padlock. The entire deployment tube is attached to the piling or support structure using U-bolts or stainless steel hose clamps. If using the latter, be certain that the drive screws are also stainless steel. The recommended configuration for this SOP calls for a customization of the sonde, which is performed by the manufacturer. Added to the sonde housing are two external bracket arms, which support the PAR sensors (Figure 16). These brackets prevent the sonde from being inserted down a standard deployment pipe (Figure 15) without additional modifications.

Figure 15. Example of a mounting pipe for YSI 6600-series sondes. The bottom of the pipe is drilled with 0.75" holes on 2.0" centers. A 0.5" bolt in installed through the bottom as a stop for the sonde. Photo courtesy of Mike Lizotte, YSI, Inc.

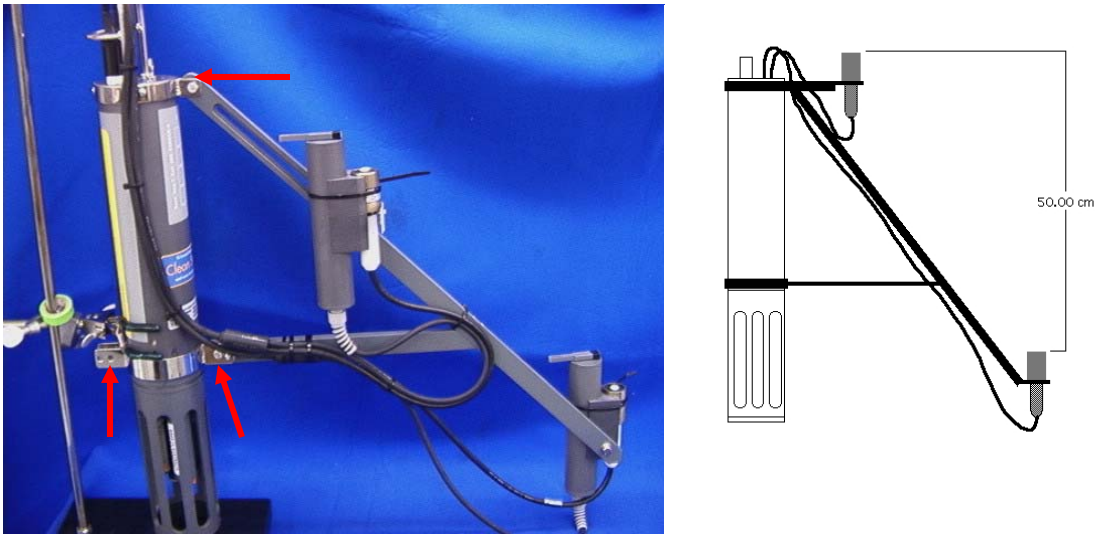

Figure 16. YSI 6600EDS modified with PAR sensors. Brackets hold the sensors 50 cm apart (in depth) and wipers clean the sensors before each reading. Photo courtesy of Mike Lizotte, YSI, Inc. Red arrows (added by the authors), point to ears on the mounting irons (see text for explanation). Note that the wiper motors in this prototype extend above the height of the LiCor PAR sensors. This has since been redesigned to avoid shading of the PAR sensors.

Three approaches are available to resolve this problem.

1) Use the general approach recommended by YSI, but cut a slot the length of the pipe to accept the PAR bracket arms.

The preferred solution for this problem is to cut a slot along the length of one side of the pipe to receive the PAR bracket arms. The slot should stop just before the perforations in the end so that the final 8-10" of pipe are fully intact (for mechanical strength). This approach provides the additional advantage of ensuring that the PAR sensors remain properly registered in a southerly orientation throughout the deployment. Note that YSI can supply the LiCor modification with either of two different style attachment irons. One style of iron has ears only on the side where the PAR bracket arms attach (single-eared). The other style has ears both on the bracket arm side and on the opposite site (double-eared). Note that in Figure 16 above, the upper mounting iron is single-eared and the lower is double-eared. When using the slotted pipe mounting approach, both irons must be of the single-eared design.

2) Lag-bolt the sonde directly to the piling using the extra ears on the mounting irons.

This approach is very straight forward, but requires that the installer get in (and under) the water. In some cases it may require SCUBA, but only mask and snorkel were needed for installation during feasibility testing at FIIS. The protection from theft provided by this method is about equal to that of option 1 since tools are required to remove the lag-bolts. Disadvantages of this method are that it provides no mechanical protection to the sonde from vandalism or accidental damage. Boats coming along side the installation may be able to rub up against and damage the sonde, particularly at low tide.

3) Use the conventional sonde deployment tube and set up a separate location to mount the PAR sensors.

The YSI sonde is still used to log the PAR data, but longer cables are used to connect the sensors to the sonde. Cables are run up the mounting tube and then down a separate bracket for the PAR sensors. Figure 17 below shows a mounting bracket used for manually wiped PAR sensors (although it is feasible to have YSI provide a custom wiring harness to power the wiper assembly). This approach is only desirable in special circumstances, such as locations that are too deep for acceptable PAR measurements or where it isn't logistically feasible to have the sonde and PAR sensors in the same location (e.g. shading from piers or potential damage from boats).

Figure 17. Separate mounting bracket for PAR sensors. The bracket is constructed of PVC conduit and fabricated to hold two upward looking LiCor PAR sensors 50 cm apart (in depth). The upper arm is shorter than the lower arm to prevent shading of the lower PAR sensor. Note that, although the bracket is painted with marine ablative antifouling paint (Interlux brand Micron-Extra™) biofouling is still present on the bracket and PAR sensors after four weeks despite weekly trips to clean the sensor (b).

The support structure should be designed so that the deployment tube can be detached and stored out of the water except when it is needed for sonde deployment (the four-week late summer index period). This will reduce the amount of servicing required to remove biofouling from its inside and outside surfaces. A fresh coat of ablative marine antifouling paint such as Interlux brand Micron-Extra™ shall be applied to all underwater surfaces of the deployment tube (inside and outside) prior to each index period. Should individual parks undertake additional monitoring using these deployment tubes, they should be scrubbed clean, inside and out, with a frequency adequate to keep them free of fouling communities, and repainted as necessary. Fouling is a particular concern in the lower, perforated portion of the deployment tube where the presence of a fouling community could affect sensor readings. For parks with severe biofouling, this problem can be reduced by omitting the bottom portion of the tube altogether. Instead, the sonde is hung from a chain so that only the sensor guard extends out the bottom of the deployment tube. A lag bolt screwed into the support structure below the deployment tube can still be used as a stop to prevent accidental loss of the instrument should the chain be dropped or the deployment tube damaged.

The deployment tube and stop-bolt must be installed so that the center of the sonde's sensor array will be positioned 0.5 m off the bottom once inserted into the tube. When deploying a sonde, it is lowered down the tube by rope or chain until it reaches the stop-bolt. A cap shall be fashioned for the top of the tube that allows it to be locked against vandalism and theft (Figure 14 a and c).

Hyperlink to Appendix 2: 13th Coast Guard District Private Aids to Navigation Information Handout

Hyperlink to Appendix 3: USCG Private Aids to Navigation Application (Form CG-2554)

2.4 SOP 4 – Continuous Water Quality Monitoring with the YSI Sonde

2.4.1 *Background and Familiarization with Instrument*

This SOP describes the methods for continuous monitoring of water quality parameters at a single station in each park during a four-week late summer index period. Data are collected using the YSI 6600EDS multi-parameter water quality monitor (sonde) deployed in autonomous mode. This specific instrument was selected based primarily upon the performance of the sonde in long- term deployments within estuaries with high levels of biofouling. Unlike other multi-parameter sondes currently on the market, the YSI 6600EDS includes wipers for each of the individual sensors. Additionally, YSI was able to customize the sonde with optical sensors, mounting brackets and wipers to allow for simultaneous measurement of underwater downwelling photosynthetically available radiation (PAR, 400-700 nm) and the PAR attenuation coefficient. It is outside the scope of this SOP to review all of the information and methods that are required to operate the YSI 6600EDS and make effective and accurate measurements. It is imperative that both field and laboratory personnel familiarize themselves with the entire YSI 6-Series Environmental Monitoring Systems manual (including the "Configuration and Deployment Instructions for YSI Model 6600EDS Sondes" supplement) and the Technical Notes and Technical Documents available from YSI at their web site and attached herein. The purpose of this SOP is to standardize instrument handling, maintenance, calibration, deployment and post-deployment procedures for all the NCBN parks. It provides step-by-step instructions as they pertain specifically to this monitoring protocol; however, it does not alone provide enough guidance to ensure consistent and accurate measurements with the YSI 6600EDS. YSI, Inc offers courses on instrument calibration and maintenance. We strongly advise that all NCBN personnel involved in instrument calibration receive annual training from YSI.

YSI, Inc. offers three different depth models of the 6600EDS: shallow (9 m), medium (61 m), and deep (200 m). Although all three models have the same resolution (0.001 m), the accuracy of the shallow water model (±2 cm) is considerably better than that of the medium depth model (±12 cm). The shallow model will be best for most NCBN parks for continuous monitoring; however, medium-depth units will still generate acceptable depth data and may substituted if a park anticipates the need for continuous monitoring at depths greater than 9 m or if it wishes to have the capability to swap units between continuous and spatial monitoring functions and the spatial survey will include stations with depths greater than 9 m. In either case, the sonde should be non-vented for pressure, and equipped with the following sensors:

- YSI 5662 Dissolved Oxygen Probe
- YSI 6136 Turbidity Probe
- YSI 6025 Chlorophyll Probe
- YSI 6560 Conductivity & Temperature Probe
- 2 LiCor LI-192SA Underwater Quantum Sensors

Note that a pH sensor is not included in the above list. The 6600EDS is routinely shipped with a pH sensor, but it should be removed from the order for a purchase credit. The

LiCor sensors are a modification to the standard 6600EDS. YSI provides a bracket to hold the two cosine corrected 2-*Pi* (flat) sensors facing upward separated by a vertical distance of 50 cm. Should the specific installation conditions warrant further modifications, it is possible to make a separate bracket for the LI-192SA sensors, and run longer cables to the 6600EDS. See the SOP on Preparation of the Continuous Logging Station.

In addition to the sonde and sensors listed above, the following equipment and supplies are also required:

- personal computer with an RS232- compliant serial port (or USB to serial converter) and running the current version of YSI EcoWatch software
- YSI 6-Series Environmental Monitoring Systems Operations Manual (including the "Configuration and Deployment Instructions for YSI Model 6600EDS Sondes" supplement), and YSI Technical Notes and Technical Documents attached herein and also at: <http://www.ysi.com/extranet/EPGKL.nsf/SearchDocs/1F24980CDDEE02AC85256CEF00763EC1!OpenDocument>
- YSI 6067B Dry Calibration Cable
- Barometer (you may use the barometer from the YSI 650MDS called for in the SOP for spatial survey monitoring).
- YSI 5775 Oxygen Probe Service Kit*
- YSI 6628 6600EDS Wiper Kit*
- YSI 6144 Wiper Pad Kit*
- Thermometer – NBS traceable with a tolerance of ± 0.2 ˚C
- 8 C-size alkaline batteries (do not substitute rechargeable batteries)
- Deionized water (ASTM type III, Laboratory Grade, or better)
- 10 mS/cm conductivity standard (YSI # 3163 or equivalent)
- Rhodamine WT Dye standard (Fluorescent FWT Red Dye - Lot# 257201, Kingscote Chemicals, 9676 N. Looney Road, Piqua, OH 45356. 800-394-0678).
- Turbidity standard of AMCO-AEPA-1® Microspheres (YSI # 6073)
- 1 Large ring stand or other home-made bracket that will allow for the 6600EDS to be suspended from its bail and hung so that the face of the turbidity sensor will be no less than 7.6 cm (3 in) from the inside bottom of the calibration cup sitting on the bench beneath it.
- Black ablative marine antifouling paint such as Interlux brand Micron-Extra™
- Laboratory glassware for preparation and dilution of standards
- LiCor LI-1400 Data Logger Instruction Manual
- LiCor LI-1400 data logger

- LiCor LI-190SA quantum sensor reserved for calibration purposes.
- LiCor LI-2003S Mounting and leveling fixture for LI-I90SA quantum sensor.
- Two LiCor LI-2222UWB underwater cables (3 m length is adequate)
- Modified 5-gal plastic bucket for transporting sonde
- Digital camera
- Calibration Logging Form (Appendix 4)

* These are expendable items provided in limited quantity with the original purchase of YSI 6600EDS sondes.

2.4.2 *Instrument Preparation*

Before the instrument can be deployed, it must be calibrated and several maintenance steps must be performed. Prior to assembling a sonde for the first time or prior to reassembling it at the beginning of each field season, the bulkhead, probe guard, and individual sensors must be painted with marine ablative antifouling paint. The recommended paint is black Interlux brand Micron-Extra™. Non-ablative paints are not recommended because the paint film will build up with repeated application and they generally lose effectiveness when left out of the water for extended periods. Note the ablative paints will rub off on the hands when the sensors are handled and can interfere with turbidity calibrations if the operator is not careful. VERY CAREFULLY follow the instructions in the "Configuration and Deployment Instructions for YSI Model 6600EDS Sondes" supplement to the YSI 6-Series Environmental Monitoring Systems manual. Sloppy application of paint may interfere with the proper function of threads on the sensors, probe guard, and sonde body, and failure to follow exactly the painting guidance from YSI may cause individual sensors to fail completely. Do not paint the sonde body itself. Instead, wrap it with plastic food wrap secured with electrical tape. After wrapping the sonde, make two holes in the wrapper at the position of the pressure sensor ports so that the wrapper does not interfere with depth measurements. This wrapper can then be removed and discarded at the end of each deployment. Assemble the individual sensors onto the sonde per instructions in the manual using extra care not to cross-thread the sensors when installing them onto the softer PVC sonde bulkhead. As a final check prior to calibration, visually inspect the sonde for any abnormalities.

Install eight fresh C-size alkaline batteries according to the directions in the YSI Operations Manual. Pay particular attention to the installation of the bottom O-ring, as this is the one that keeps the battery compartment from leaking. It must be properly seated in its groove on the bottom of the battery lid, clean and free from contaminants, and lubricated with a VERY light film of clean silicone grease. The O-ring on the side of the battery lid seals out contaminants and fouling material and should also be cleaned and lubricated with a light film of clean silicone grease. Also note that over tightening the battery lid screws may distort the lid and cause the battery compartment to flood.

2.4.3 *Pre-Deployment Calibration Methods*

2.4.3.1 *LiCor 192SA PAR sensors*

Although LiCor recommends biennial factory calibration of its LI-192SA quantum sensors, we recommend here that all sensors used for continuous underwater deployments be factory calibrated annually. The diffuser lens of the LI-192SA is made of acrylic, which is very slightly permeable to water vapor. With long-term deployments, excessive water vapor inside the sensor has been implicated in very occasional sensor failure. Careful pre- and post-deployment calibration checks will assure data quality. LiCor sensors that are used to make discrete light profiles for this SOP and for the spatial survey SOP may be factory calibrated every two years. Confirm the pre-deployment calibration of both LI-192SA underwater sensors for this SOP by comparing their output to that of a third, recently calibrated sensor reserved specifically for this purpose. A LI-190SA "deck cell" will work well for this purpose.

i. Attach the two underwater quantum sensors and the third "calibration sensor" to channels I1, I2 and I3 of a LiCor LI-1400 data logger (using appropriate underwater cables for the underwater sensors).

ii. Mount the sensors to a bracket that will keep all three of them aligned with their diffuser elements horizontal.

iii. Program the LI-1400 to log the output from all three sensors (channels I1, I2 and I3) for duration of one hour with the sampling period set to one second and the logging period set to one hour. Follow instructions in the LI-1400 Instruction Manual for programming the data logger. For the underwater quantum sensors, use the "in air" multipliers from their most recent LiCor calibration certificates.

iv. Place the sensors in full sunlight within 2 hours of local apparent noon. Keep them there for the duration of the logging period.

v. At the end of the one hour logging period, record the one-hour average value for each of the LI-192SA sensors on the calibration log. (Follow instructions in the LI-1400 Instruction Manual for viewing logged data.)

2.4.3.2 *Standard solutions*

2.4.3.2.1 Deionized Water

Deionized water is required for calibration of many of the sensors so some guidance is provided here on the quality of water that should be used for these purposes. In general it is recommended that water be purchased or purified on site to a quality equal to or better than ASTM standards for Type III (Laboratory Grade) water. Note that a USGS sampling of supermarket distilled waters from around the country found conductivities falling well outside the standard for even ASTM Type III Laboratory Grade water (USGS Office of Water Quality Technical Memorandum 92.01). While not part of this protocol, some of the network parks already include the measurement of pH among their suite of monitoring variables. ASTM Type II (Reagent Grade) water should be used for the mixing of buffer solutions. This SOP calls for the use of pre-prepared conductivity standards for calibration of the sonde. Nevertheless, you should be further deterred from

preparing or diluting your own standards by the fact that even ASTM Type II water is inadequate for these purposes. Water prepared by distillation or ion exchange should include a polishing step by passing it through a 0.45µm filter to remove some bacteria and any ion exchange medium that escapes the columns. This is particularly relevant for calibration of the turbidity sensor (EPA Method 180.1).

2.4.3.2.2 Turbidity standard

The recommended standard for this SOP is a 123 nephelometric turbidity unit (NTU) standard made of AMCO-AEPA-1® Microspheres prepared specifically for the YSI 6136 turbidity probe (YSI part number 006073). This standard can only be obtained from YSI or its dealers. Formazin based turbidity standards are less expense and are also effective, but are not recommended for the NCBN protocol. One of the reagents used in its preparation, hydrazine sulfate, is a carcinogen and consequently requires special care in handling and disposal. Even prepared formazin suspensions can contain residual hydrazine sulfate. In addition, formazin quickly settles out of suspension and may be less stable in dilute form.

2.4.3.2.3 Chlorophyll standards

This SOP calls for a two-step process in calibrating the fluorometric output from the YSI 6025 Chlorophyll Sensor to actual chlorophyll-*a* concentrations (in µg/l). The first calibration step, using Rhotamine WT dye, produces approximate values and allows for sensor drift to be evaluated over the deployment period. The second calibration step yields final reportable concentrations. This second step involves creating a standard curve between values reported by the YSI 6025 Chlorophyll Sensor and values determined by a laboratory method where phytoplankton cells are collected on a filter, cell membranes are ruptured with acetone, and chlorophyll is measured spectrophotometrically. The calibration standard described here is for the first calibration step (Rhotamine WT dye standard). Chlorophyll extraction methods for the second calibration step are covered under a separate SOP (see SOP on Chlorophyll Methods).

Rhodamine WT Dye comes from the manufacturer as an approximate 2% stock solution (Fluorescent FWT Red Dye - Lot# 257201, Kingscote Chemicals, 9676 N. Looney Road, Piqua, OH 45356. 800-394-0678). Since the fluorescence of Rhodamine WT shows an inverse relationship with temperature, this effect must be accounted for when checking for sensor drift. YSI has prepared a temperature compensation chart for this specific lot number that relates a 1:40,000 dilution of the stock dye to approximate chlorophyll-*a* units (in µg/l). Consequently, it is important that you use this same lot number of Fluorescent FWT Red Dye. If this lot number becomes unavailable, then a new temperature compensation relationship will need to be established. Only a very small amount of dye is needed for the calibration, however, so provided that the stock bottle is kept well sealed to prevent evaporation and kept in the dark, it should last for many years.

Each field season, prepare a fresh concentrated working standard from 2% Fluorescent FWT Red Dye. This will be a 1:200 dilution from the 2% stock solution (or approximate 100 mg/l). Use this working standard for one field season only. To prepare the 1:200 dilution, use a volumetric pipette to accurately transfer 2.5 ml of 2% Fluorescent FWT Red Dye into a 500 ml volumetric flask. Fill the flask to the volumetric mark with

deionized water and mix. Transfer this concentrated standard to a glass amber bottle, and store tightly capped in a laboratory refrigerator to retard decomposition. The bottle of stock 2% Fluorescent FWT Red Dye should also be recapped tightly, sealed with Parafilm M®, and stored in the refrigerator.

To calibrate the sonde, prepare a working standard of approximately 0.5 mg/l by mixing a 1:200 dilution of the concentrated working standard. This will be a 1:40,000 dilution of the stock dye solution. Accurately transfer 2.5 ml of the concentrated working standard into a 500 ml volumetric flask. Fill the flask to the volumetric mark with LAB TEMPERATURE deionized water and mix. Transfer this working standard to a glass amber bottle and use immediately. If kept tightly capped and refrigerated, this dilute standard can be stored for up to 24 hours; however it must be brought back to lab temperature to perform calibrations.

2.4.3.3 *6600 EDS Calibration*

2.4.3.3.1 General notes

During the calibration procedure, never accept any calibrations that have produced a warning message. You must instead determine the cause of the problem, correct it, and recalibrate following this SOP and the instrument manual.

Prior to each deployment, batteries, dissolved oxygen membranes, and wiper pads for the turbidity and chlorophyll probes must all be replaced with new ones. During the calibration procedure, use a standard wiper on the YSI 6136 turbidity probe. This must be replaced with an EDS turbidity wiper assembly (with brush) prior to final deployment. The EDS 6628 wiper + brush assembly on the sonde and the brushes on the LiCor PAR sensors must be examined and replaced if they are excessively worn. Pay particular attention to YSI guidance on appropriate clearances for wiper assemblies since excessively tight installation could slow down or stall the wiper motor.

Dissolved oxygen membranes are unstable for the first 3 to 6 hours after they are installed. This SOP calls for the DO membrane to be replaced and the sonde calibrated 6-12 hours before deployment. It is then put into autonomous/logging mode and allowed to collect 6-12 hours of data while still in the laboratory. After this stabilization period, just the DO sensor is recalibrated, and the sonde is ready for deployment. The advantage of this approach is that it allows the DO probe to fully stabilize while providing additional data prior to deployment. This can aid in future diagnosis of any invalid data.

Except where explicitly noted in the SOP, standards should NOT be used for more than one calibration. They may, however, be saved and used to pre-rinse the sensors and the calibration cup before calibration with fresh standard solutions. Rather than measure calibration solutions with graduated cylinders each time they are dispensed, you may choose to mark the outside of the calibration cup at the appropriate levels for each probe. Dispensing standards directly into the calibration cup will also help prevent contamination from dirty glassware. For many of the calibration steps, reaching a stable calibration point requires the probe and calibration solution to thermally equilibrate. This occurs more quickly if both the sonde and all the calibration solutions are at laboratory temperature. Be sure to prepare deionized water in advance and store an ample supply in a carboy.

The 6600EDS should be calibrated while hanging from a ring stand or custom-mounted hook. Height is critical for proper calibration of the turbidity and chlorophyll sensors. The bottom face of the 6136 Turbidity Probe should be set so there is at least 7.6 cm of clearance between it and the inside bottom of the calibration cup (when unthreaded from the sonde body). This is the minimum clearance in order to avoid backscatter interference when calibrating the turbidity and chlorophyll sensors. Greater clearances will result in increased consumption of standard solutions. The conductivity probe can, however, be calibrated upside down in order to conserve calibration solution.

The wiper assemblies on both the turbidity and chlorophyll probes must "park" 180˚ away from the optical windows on these probes. Actuating the wipers while in air can sometimes cause them to park in different locations. At several points in the calibration procedure you are prompted to confirm that the wipers are parking properly. If they fail to do so, troubleshoot using guidance in the YSI 6-Series Operations Manual.

Taking the above notes into account, the following calibration procedure should be completed in the order it is presented.

2.4.3.3.2 Dissolved Oxygen

i. Inspect the DO probe anodes. If they are darkened or gray in color, recondition using the 6035 reconditioning kit.

ii. Install a new membrane, making sure that it is tightly stretch and wrinkle free.

iii. Hang the sonde from the ring stand or custom support bracket so that the turbidity probe is on the left, and the chlorophyll probe on the right. Put approximately 0.5 cm of water in the bottom of the calibration cup and loosely attach it to the sonde (engaging several threads). Attach the Dry Calibration Cable between the sonde and computer, launch EcoWatch software, and establish communication with the sonde.

iv. Go to the sonde <Report> menu and enable the <DO Charge> option. Then go to the "<Run> menu and start the sonde in the <Discrete Sample> mode with a 4 second sample interval. Allow the sonde to run for 10 minutes to "burn in" the membrane.

v. After the burn-in, go to the sonde <Advanced> <Setup> menu and enable the "RS-232 auto-sleep" function. Wait 60 seconds before proceeding.

vi. Perform a High-to-Low Transmission Test: Start the sonde in <Discrete Sample> mode with a 4 second sample interval. Disregard the first two DO % Saturation samples, and then record the next 10 samples. The DO % Saturation values must start high and drop with each 4-second reading. It does not matter if the readings do not reach 100%, but there must be a high to low trend. If the recorded values start low and then climb upward, the sensor has a problem and must be reconditioned or replaced. Record the 10 readings and the pass-fail status of the test on the calibration log. Escape from the <Run> mode.

vii. Wait at least 5 minutes in the idle (not <Run>) mode before proceeding. You must also wait at least 10 minutes from the time the calibration cup (with 0.5 cm water in bottom) was last attached to the sonde body. Once these minimum times

have been reached, calibrate the DO sensor using the <% Saturation> method. Be sure to check and enter the current barometric pressure. Note that laboratory barometer readings are usually "true" values of air pressure and can be used "as is" for oxygen calibration. The barometer on the 650MDS (if properly calibrated) also reports "true" barometric pressure and can be used "as is." Pressure reported by the National Weather Service, however, is usually "corrected" to sea level and cannot be used in raw form. See the YSI Operations Manual for correction methods. Record the DO Charge on the calibration log. It should be 50 ± 25.

viii. When the calibration has been accepted, check the "DO gain" for the sensor and record it on the calibration log. This is found under the sonde's <Advanced Menu> under the <Cal Constants> option. The target value for gain is 1.0, with an acceptable range from 0.7 to 1.4.

2.4.3.3.3 Conductivity

i. You will need approximately 425 ml of conductivity standard to calibrate this probe. This volume can be reduced to approximately 225 ml if the sonde is held upside down while performing the calibration.

ii. Calibration of the conductivity probe is very vulnerable to contamination. Before performing the calibration, the calibration cup and all the surfaces of all the probes must be triple rinsed with a small amount of conductivity standard. Used standard from previous execution of this SOP is acceptable.

iii. Pour approximately 50 ml of 10 mS/cm conductivity standard into a clean and dry calibration cup and fully attach it to the sonde body. Swirl, tip and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution (do not re-use rinse standards). Repeat this step two more times.

iv. Pour 425 ml of FRESH 10 mS/cm conductivity standard into the calibration cup and attach it to the sonde body (225 ml if calibrating the sonde upside down).

v. Insure that the conductivity probe is completely submerged in the standard. The hole in the side of the probe must be under the surface of the solution and not have any trapped bubbles in the opening.

vi. Initiate calibration of the probe by selecting the <specific conductance> option and entering "10" for the conductivity of the standard (Be sure to enter the conductivity of the standard in mS/cm, not µS/cm).

vii. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde, the conductivity standard and the deionized water from previous steps are not all at the same laboratory temperature). Once a stable reading has been maintained for approximately 30 seconds, accept the calibration (press <enter>) and record on the calibration log the last value reported prior to doing so. With an NBS-traceable thermometer, verify the accuracy of the sonde temperature reading and record it on the calibration log.

viii. When the calibration has been accepted, check the "Conductivity Cell Constant" for the sensor and record it on the calibration log. This is found under the sonde's

<Advanced Menu> under the <Cal Constants> option. The target value for this probe is 5.0 ± 0.45. Numbers outside this range usually indicate a problem in the calibration process or a contaminated standard. Never override a calibration error message and investigate any "Out of Range" report.

2.4.3.3.4 Chlorophyll

i. You will need deionized water and a working concentration of Rhodamine WT dye standard to calibrate the chlorophyll fluorometer. Prepare the working Rhodamine standard following the procedures in section 2.4.3.2.3.

ii. Half fill the calibration cup with deionized water and attach it to the sonde body. Swirl, tip and gently shake the sonde in order to thoroughly wet all of the probe and bulkhead surfaces. Discard this rinse water and repeat two more times.

iii. Fill the calibration cup with at least 180 ml of fresh deionized water and set it on the bench below the sonde. Do not attach it to the sonde body. The distance between the face of the turbidity probe and the inside bottom of the calibration cup should be no less than 7.6 cm.

iv. Select the <2 point > method from chlorophyll calibration menu, and enter zero <0> as the value for the first point. Start the calibration routine and actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

v. If the deionized water is already at laboratory temperature wait approximately 1 minute for thermal equilibration. Once a stable reading has been maintained for approximately 30 seconds, press <enter> to set the calibration. On the calibration log, record the last value displayed before pressing <enter>. Note: chlorophyll values tend to bounce more than other values, this is normal.

vi. Pour approximately 50 ml of Rhotamine WT working standard into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.

vii. Fill the calibration cup with at least 180 ml of fresh Rhotamine WT working standard and set it on the bench below the sonde. Do not attach it to the sonde body.

viii. Confirm that the standard is at laboratory temperature, then use the temperature compensation chart to select the proper chlorophyll concentration to enter as the second point in the calibration from Table 2 of YSI Technical Note: Alternate Calibration Methods for the YSI 6025 Chlorophyll Sensor Appendix 5).

ix. Continue the calibration routine and actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

x. Allow the sonde to run until a stable reading is maintained for approximately 30 seconds, then press <enter> to set the calibration. On the calibration log, record

the last value displayed before pressing <enter>. Rinse probes with DI water and pat dry with Kimwipes®.

xi. When the calibration has been accepted, check the "Chlorophyll Offset" for the sensor and record it on the calibration log. This is found under the sonde's <Advanced Menu> under the <Cal Constants> option.

2.4.3.3.5 Turbidity

i. The sonde and calibration cup must be clean or contamination of the zero NTU standard will occur. Half fill the calibration cup with deionized water and attach it to the sonde body. Swirl, tip and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this discard this rinse water and repeat two more times.

ii. Fill the calibration cup with at least 180 ml of fresh deionized water and set it on the bench below the sonde. Do not attach it to the sonde body. The distance between the face of the turbidity probe and the inside bottom of the calibration cup should be no less than 7.6 cm.

iii. Select the <2 point > method from turbidity calibration menu, and enter zero <0> as the value for the first point. Start the calibration routine and actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

iv. Wait for a stable reading to be maintained for approximately 30 seconds, then press <enter> to set the calibration. The reading on-screen should now be approximately 0.0. On the calibration log, record the last value displayed before pressing <enter>.

v. Pour approximately 50 ml of turbidity standard (used is acceptable) into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.

xii. Fill the calibration cup with at least 180 ml of fresh turbidity standard and set it on the bench below the sonde. Do not attach it to the sonde body.

xiii. Continue the calibration routine entering 123 NTU for the second standard. (NOTE: the recommended AMCO-AEPA-1® standard has a different value depending upon the sensor. It is 123 NTU for the YSI 6136.) Actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

xiv. Allow the sonde to run until a stable reading is maintained for approximately 30 seconds, then press <enter> to set the calibration. On the calibration log, record the last value displayed before pressing <enter>.

xv. When the calibration has been accepted, check the "Turbidity Offset" for the sensor and record it on the calibration log. This is found under the sonde's <Advanced Menu> under the <Cal Constants> option.

xvi. Rinse probes with deionized water, put approximately 0.5 cm of water in the bottom of the calibration cup and loosely attach it to the sonde (engaging several threads).

2.4.3.3.6 Pressure/Depth calibration

i. From the <Calibrate> menu, select <Pressure-Abs>.

ii. Input 0.00 (or a known sensor offset in feet if working far from sea level).

iii. Wait until no significant change occurs for approximately 30 seconds, then press <enter> to confirm the calibration.

2.4.3.3.7 LiCor 192SA PAR sensor calibration

Confirm that the procedures for checking the calibration of the LiCor PAR sensors have been performed and recorded in the calibration log (section 2.4.3.1), then attach them to the PAR sensor mounting-bracket on the YSI. In the <Advanced> menu, under <Sensor>, confirm that the correct LiCor calibration multipliers have been entered for each of the PAR sensors, and that the sensors have not been inadvertently swapped between the upper and lower bracket arms. On the calibration log, record the LI-192SA serial numbers, their positions on the sonde, the calibration multipliers used, and the date of the last manufacturer's calibration.

2.4.3.3.8 Programming and miscellaneous preparations for deployment

Replace the standard wiper on the 6136 turbidity probe with the 6628 EDS wiper assembly and brush. Pay particular attention to YSI guidance on appropriate clearances for this wiper assembly since excessively tight installation can slow down or stall the wiper motor. Put approximately 0.5 cm of water in the bottom of the calibration cup and attach it to the sonde.

Check to confirm that there are no remaining data (.dat) files stored in the sonde's memory. These should previously have been removed from the sonde immediately after being downloaded to computer, so their presence will indicate a failure to follow the SOPs of this protocol. Proceed with caution! Download any files to a specially labeled directory and annotate the calibration log to this effect. Use EcoWatch to open the date files and examine them to ensure they have not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data files and confirm that they too are readable using EcoWatch. After these steps have been completed, reestablish communication with the sonde, select the <File> menu and <Delete all files>.

Program the sonde with the correct date and LOCAL TIME. For all anticipated use of this SOP, this will be Eastern Daylight Savings Time. The following report parameters should be enabled in the <Report> menu: date, time, temperature, specific conductance, salinity, dissolved oxygen saturation, dissolved oxygen concentration, DO charge, depth, turbidity, chlorophyll, battery voltage, PAR1 and PAR2.

Select <Run> from the sonde's main menu and initiate <unattended sample> mode. The next window will prompt you to enter the sampling interval (15 minutes), the start date (enter today's date), and the start time (enter the current time rounded up to the next

quarter hour). Keep the sampling duration at the default (365 days) and confirm that battery life and free memory are adequate to complete the intended duration of deployment.

The eight-character naming convention for the data file is:

- Station identifier for when a park has more than one logging station (one digit, using "1" as the default for parks with only one station).
- NPS park code (4 characters)
- Year (last two digits)
- Deployment sequence - used for when new sondes are swapped in or serviced due to biofouling (1 character using sequential lettering)

Example: 1CACO04A

Site names may be more descriptive (up to 31 characters), but should start with the same first 5 characters as the data file.

Activate logging. The sonde will now record data for the water-saturated air environment within the calibration cup for the 6 to 12 hours before receiving a final dissolved oxygen calibration and being deployed. Confirm that the sonde is properly logging data by going to the <Status> menu and making sure that the sonde returns a "Logging Active" message.

2.4.3.3.9 Final dissolved oxygen calibration

Just before bringing the sonde into the field for deployment, and after collecting 6 to 12 hours of pre-deployment data in the laboratory, perform a final calibration of the dissolved oxygen probe. It is not necessary to exit the unattended mode to do this. Simply go to the <calibrate> menu and repeat the last 2 steps from section 2.4.3.3.2 on page 64. This re-calibration information will now be part of the data record and will confirm proper operation prior to the deployment. Confirm that the sonde is properly logging data by going to the <Status> menu and making sure that the sonde returns a "Logging Active" message.

2.4.4 *Deployment*

i. Before leaving the laboratory, remove the calibration cable from the sonde body and install the connector cap

ii. Remove the calibration cup from the sonde body and install the sensor guard. It is best not to attempt this in the field since the oxygen membrane is vulnerable to being damaged if it is bumped by the guard.

iii. Wrap the sonde in a white towel that has been soaked in tap water. The towel should cover the entire sensor guard and go around it at least twice. This will provide a humid environment for the sensors, protection from thermal extremes, and some degree of shock protection.

iv. Transport the sonde to the field in a specially-modified white 5-gallon plastic bucket. To prepare the bucket, place approximately 8 kg of lead weight in its

bottom. Neoprene-covered soft weight packs containing lead shot that are used by SCUBA divers are excellent because they will not risk damaging the sonde. Also put approximately 2 cm of tap water in the bottom of the bucket. Cut a hole in the middle of the lid that will accommodate the sonde body wrapped in the towel. The lid will support the sonde, help to maintain the humid environment, and keep water from spilling or sloshing out. As necessary, partially disassemble the bracket arms holding the LiCor sensors so that the assembly rests securely in the bucket. Avoid any contact with the PAR wipers or damage may result.

v. As much as possible, keep the sonde & bucket out of direct sunlight to prevent thermal extremes.

vi. Plan the deployment so that it occurs within 2 hours either side of local apparent noon (for most of the Parks this will be close to 13:00 hrs due to daylight savings time).

vii. Deploy the sonde at the logging station in a manner consistent with the particular mounting bracket design (see SOP on Preparation of the Logging Station). Record the date and time of the deployment, the water depth at the time of deployment, and the distance between the substratum and the bottom of the sensor guard. This should be either 1.00 m or 0.50 m depending upon the park.

viii. The sonde will take measurements in increments of 15 minutes on the quarter hour. All other measurements made alongside the sonde should also be taken on the quarter hour so that results can be directly compared.

ix. At one such time, make an independent measurement of PAR attenuation using the Spatial Survey Methods SOP. Make sure that neither the LiCor PAR profiling apparatus nor the YSI sonde are shaded by the boat, the logging installation, or personnel.

x. In a clean 500 ml amber polyethylene bottle, collect a water sample for chlorophyll analysis by extractive methods. The sample should be collected at the same depth as the sonde probes, and close in time to a logging event (on the quarter hour). Follow the instructions in SOP 5 (Spatial Water Quality Monitoring with the YSI Sonde) for collection and handling of this water sample, and the instructions in SOP 7 (Chlorophyll-*a* Sampling and Analysis) for laboratory analysis.

2.4.5 *Routine Service of the Sonde*

In order to calibrate the chlorophyll sensor, discrete samples of chlorophyll must be gathered at regular intervals for analysis by extractive methods. The continuous monitoring station must also be visited regularly to make separate and independent measurement of the other monitoring metrics. The minimum frequency for this sample collection is weekly, and it shall be accomplished by incorporating a visit to the logging station into the weekly spatial survey (see Spatial Survey Methods SOP). Grab samples for extractive chlorophyll-*a* analysis from this site are used to calibrate the logging chlorophyll-a sensor, while data from the spatial survey YSI sonde and LiCor PAR profiler are used to post-checking all the continuous sensors and correct for any sensor drift. Under anticipated conditions in NACP, the YSI 6600EDS can be expected to

remain free from excessive biofouling and functionally operative for the duration of the index period. This will yield a total of five discrete extractions of chlorophyll against which to calibrate the YSI chlorophyll sensor. At some of the network parks, however, high rates of fouling will require that the sonde be replaced once during the four-week deployment period (at the end of the second week). For these parks, weekly samples at the logging station would yield only three discrete samples for calibration of the chlorophyll sensor. In this case, supplemental samples must be gathered either by making additional dedicated trips to the logging station or by making multiple stops at the logging station at different times of day during the four weekly spatial surveys. Perform the following steps for each of these routine service visits. The YSI Calibration Log form contains fields for entering data and information related to the deployment, servicing and retrieval of the sonde (do not bring original copies into the field).

i. The sonde will take measurements in increments of 15 minutes on the quarter hour. All other measurements made alongside the sonde should also be taken on the quarter hour so that results can be directly compared.

ii. At one such time, make an independent measurement of PAR attenuation using the Spatial Survey Methods SOP. Make sure that neither the LiCor PAR profiling apparatus nor the YSI sonde are shaded by the boat, the logging installation, or personnel.

iii. In a clean 500 ml amber polyethylene bottle, collect a water sample for chlorophyll analysis by extractive methods. The sample should be collected at the same depth as the sonde probes, and close in time to a logging event (on the quarter hour). Follow the instructions in SOP 5 (Spatial Water Quality Monitoring with the YSI Sonde) for collection and handling of this water sample, and the instructions in SOP 7 (Chlorophyll-*a* Sampling and Analysis) for laboratory analysis.

iv. Lower a second YSI sonde (as prepared for the spatial survey) to the same depth as the logging sonde. Actuate the wipers on the second YSI and initiate logging. Continue logging until you have captured data for the period coinciding with a logging event on the original sonde (which measures on the quarter hour). This can be accomplished by either allowing the second sonde to log for at least 15 full minutes, or by specifically targeting the quarter-hour event. If attempting the latter, be certain to log for at least 2 minutes on either side of the quarter hour. These data will be used to check for drift of the logging YSI sonde within the index period deployment.

2.4.6 *Swapping a Sonde During the Index Period*

For parks where the presence of extreme biofouling requires that the sonde be swapped out during the index period, use the following procedures to accomplish the task. This should occur during the middle of the index period (at the end of the second week). The preferred method is to prepare and calibrate a second sonde and deploy it BEFORE removing the first sonde. This will allow for a continuous data record for the entire index period and aid in the interpretation of any sensor drift. If this is not feasible, the existing sonde should be retrieved, serviced, recalibrated, and returned to the field within the shortest time possible (24 hours). The YSI Calibration Log form contains fields for

entering data and information related to the deployment, servicing and retrieval of the sonde (do not bring original copies of the calibration log into the field).

i. The day before it will be swapped into service, a second YSI 6600EDS should be calibrated following the methods of this SOP.

ii. Plan the deployment/swap so that it occurs within 2 hours either side of local apparent noon (for most of the Parks this will be close to 13:00 hrs due to daylight savings time).

iii. When exchanging the old sonde with the new, they should be allowed to collect data at the same depth for at least one logging cycle. This can be accomplished by suspending the new sonde from the logging station at a depth equal to that of the existing sonde. Wait until just after the quarter hour to ensure that both sondes have logged a sampling event. The following two steps need to be accomplished close in time to a logging event, so two logging intervals may be needed.

iv. At the same time as a logging event, make an independent measurement of PAR attenuation using the Spatial Survey Methods SOP. Make sure that neither the LiCor PAR profiling apparatus nor the YSI sonde being retrieved are shaded by the boat, the logging installation, or personnel. (Note: This profile is used to post-calibrate PAR attenuation on the existing sonde only. At this time the sonde being newly deployed may not hang at a proper angle for making good measurements of PAR attenuation. A second discrete PAR profile will be required once the new sonde is in its final position.)

v. Also at the same time as a logging event, use a clean 500 ml amber polyethylene bottle to collect a water sample for chlorophyll analysis by extractive methods. The sample should be collected at the same depth as the sonde probes. Follow the instructions in SOP 5 (Spatial Water Quality Monitoring with the YSI Sonde) for collection and handling of this water sample, and the instructions in SOP 7 (Chlorophyll-*a* Sampling and Analysis) for laboratory analysis.

vi. Remove the existing YSI sonde from its mounting bracket and promptly swap in the new sonde. Take several digital pictures of the LiCor sensors and the sonde sensors. This photo documentation should be a permanent part of the record. Partially disassemble the LiCor sensor bracket to the extent necessary for transportation, and then wrap the sonde in a white towel that has been soaked in tap water. The towel should cover the entire sonde and go around the body at least twice. Transport the sonde back to the lab in the modified white 5-gallon plastic bucket keeping the sonde and bucket out of direct sunlight to prevent thermal extremes. The optical windows on the LiCor sensors should be protected from abrasion and from drying out by putting some water in the annular ring around the optical window and then capping them with the covers provided by the manufacturer.

vii. At the same time as a logging event, make an independent measurement of PAR attenuation using the instructions in SOP 6 (Spatial Water Quality Monitoring with LiCor PAR Instruments). Make sure that neither the LiCor PAR profiling

apparatus nor the YSI sonde just deployed are shaded by the boat, the logging installation, or personnel. Note: You are now assured proper positioning of the newly deployed YSI sonde and LiCor PAR sensors. This is the PAR profile that should be used when post-calibrating the newly-deployed YSI sonde for PAR attenuation.

2.4.7 *Final Retrieval of the Sonde*

Methods for final retrieval of the sonde are similar to the above section (2.4.6 -Swapping a Sonde During the Index Period), except that there is no need to prepare and deploy a new YSI 6600EDS. Simply omit steps that pertain to deployment of a second sonde. Keep the sonde in logging mode until back in the lab and preparing to perform the post-deployment calibration check.

2.4.8 *Post-Deployment Calibration Check*

2.4.8.1.1 General notes

In the laboratory, a post-deployment calibration check must be performed on the oxygen, conductivity, and depth probes BEFORE they are cleaned and on the turbidity and chlorophyll probes AFTER they are cleaned (to prevent contamination of the 0.0 NTU and 0 µg/l standards). During the calibration check, keep the YSI 6628 EDS wiper (wiper + brush) on the turbidity probe for the first part of the calibration check (dissolved oxygen, conductivity and depth). At the time the sonde is cleaned, the YSI 6628 EDS wiper should be replace with a standard wiper. Pay particular attention to YSI guidance on appropriate clearances for these wiper assemblies since excessively tight installation will scratch and damage the optical windows on these sensors.

Post-deployment calibration checks of the 6600EDS should be performed with it hanging from a ring stand or custom-mounted hook. Height is critical for calibrating the chlorophyll and turbidity sensors. The bottom face of the 6136 Turbidity Probe should be set so there is at least 7.6 cm of clearance between it and the inside bottom of the calibration cup (when unthreaded from the sonde body). This is the minimum clearance in order to avoid backscatter interference when calibrating the turbidity and chlorophyll sensors. Greater clearances will result in increased consumption of standard solutions.

Except where explicitly noted in the SOP, standards should NOT be re-used for more than one calibration (or calibration check). Used standards may, however, be saved to pre-rinse sensors and the calibration cup prior to putting them into fresh standard solutions. Taking the above notes into account, the following calibration check should be completed in the order presented.

2.4.8.1.2 Sonde Preparation

i. Before checking the calibration of the sonde, it is acceptable to pre-clean the sonde body by removing the plastic wrap. If, however, there is significant fouling around the pressure sensor ports, it is best to leave the wrapper in place until after the depth calibration has been checked because changes in this fouling may affect the pressure readings.

ii. Remove the sensor guard from the sonde body and inspect all of the sensors without dislodging any fouling material. In particular, note the positions and condition of the wipers, the degree of biofouling on each of the sensors, and the condition of the membrane on the dissolved oxygen sensor. It is best not to replace the sensor guard with the calibration cup in the field since the oxygen membrane is vulnerable to being damaged if it bumped by the guard or the calibration cup. The wet-towel method will provide better protection to the sonde and sensors.

iii. Hang the sonde from the ring stand or custom support bracket so that the turbidity probe is on the left, and the chlorophyll probe on the right. Put approximately 0.5 cm of water in the bottom of the calibration cup and loosely attach it to the sonde (engaging several threads). Attach the Dry Calibration Cable between the sonde and computer, launch EcoWatch software, and establish communication with the sonde.

iv. Take the sonde out of logging mode. From the sonde's <Run> menu select <unattended sample> then select "stop logging."

v. Allow adequate time for the air in the calibration cup to become saturated with water vapor and for the sonde temperature to stabilize (15 minutes to 2 hours depending upon temperature differential).

2.4.8.1.3 Dissolved Oxygen

i. Confirm that the sonde is still properly logging data by going to the <Status> menu and making sure that the sonde returns a "Logging Active" message.

ii. Go to the sonde <Report> menu and enable the <DO Charge> option. Then go to the <Run> menu and switch the sonde to <Discrete Sample> mode. Set the sampling interval to 4 seconds and activate sampling.

iii. DO High-to-Low Transmission Test: Start the sonde in <Discrete Sample> mode with a 4 second sample interval. Disregard the first two DO % Saturation samples, and then record the next 10 samples. The DO % Saturation values must start high and drop with each 4-second reading. There must be a high to low trend. If the recorded values start low then climb upward, the sensor has a problem. Record the 10 readings and the pass-fail status of the test on the calibration log.

iv. Allow the DO % Saturation value to stabilize, and then record this value in the calibration log along with the value for DO charge and the current barometric pressure.

v. Escape from the <Run> mode.

2.4.8.1.4 Conductivity

i. You will need approximately 425 ml of conductivity standard to check the calibration of this probe. Because conductivity is very vulnerable to contamination, the "upside down" method available for the initial calibration is not recommended because it may dislodge additional fouling material.

ii. Before performing the calibration check, the calibration cup and all the surfaces of all the probes must be triple rinsed with a small amount of conductivity standard. Used standard from previous execution of this SOP is acceptable.

iii. Pour approximately 150 ml of 10 mS/cm conductivity standard into a clean and dry calibration cup and attach it to the sonde body. Gently tip the sonde to thoroughly wet all of the probe and bulkhead surfaces. Avoid aggressive shaking or swirling that can dislodge fouling material. Discard this standard solution (do not re-use rinse standards) and repeat two more times.

iv. Pour 425 ml of FRESH 10 mS/cm conductivity standard into the calibration cup and attach it to the sonde body.

v. Insure that the conductivity probe is completely submerged in the standard. The hole in the side of the probe must be under the surface of the solution and not have any trapped bubbles in the opening.

vi. Go to the <Run> menu and activate <Discrete Sample> mode.

vii. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde, the conductivity standard is not all at the same laboratory temperature). Once the reading has stabilized, record it in the calibration log.

viii. With an NBS-traceable thermometer, verify the accuracy of the sonde temperature reading and record it on the calibration log.

ix. Escape from the <Run> mode.

2.4.8.1.5 Pressure/Depth calibration

i. Go to the <Run> menu and activate <Discrete Sample> mode.

ii. Once the reading has stabilized, record it in the calibration log.

iii. Escape from the <Run> mode.

2.4.8.1.6 Cleaning of the YSI EDS6600 sonde and replacement of wipers

Clean the YSI sonde following guidance in the YSI Environmental Monitoring Systems Operations Manual. Do NOT clean the LiCor PAR sensors at this time. A soft tooth brush may be useful for removing fouling material. Use caution with abrasive cleaning agents (scouring pads) or sharp objects (dental picks), which may scratch and damage the optical windows on chlorophyll and turbidity sensors. Use a syringe to clean the pressure sensor by repeatedly rinsing clean water through the port. Replace the YSI 6628 EDS wiper + brush assembly with a standard turbidity probe wiper. Also replace the wiper pad on the chlorophyll sensor. Pay particular attention to YSI guidance on appropriate clearances for these wiper assemblies since excessively tight installation will scratch and damage the optical windows on these sensors.

Hang the sonde from the ring stand or custom support bracket so that the turbidity probe is on the left, and the chlorophyll probe on the right. Attach the Dry Calibration Cable between the sonde and computer, launch EcoWatch software, and establish communication with the sonde.

2.4.8.1.7 Chlorophyll and Turbidity

i. You will need deionized water, AMCO-AEPA-1® turbidity standard, and a working concentration of Rhodamine WT dye standard to check the calibration of the chlorophyll and turbidity probes. Prepare the working Rhodamine standard following the procedures in section 2.4.3.2.3 on page 65.

ii. Half fill the calibration cup with deionized water and attach it to the sonde body. Swirl, tip and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this discard this rinse water and repeat two more times.

iii. Fill the calibration cup with at least 180 ml of fresh deionized water and set it on the bench below the sonde. Do not attach it to the sonde body. The distance between the face of the turbidity probe and the inside bottom of the calibration cup should be no less than 7.6 cm.

iv. Go to the <Run> menu and activate <Discrete Sample> mode with a 4-second sample interval.

v. Actuate the wipers to remove any air bubbles from the face of the probes (press<3>). Confirm that the wiper is parked 180º away from the optical windows on the probe faces.

vi. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde and deionized water are not all at the same laboratory temperature). Once the readings for chlorophyll and turbidity have stabilized, record them in the calibration log.

vii. Escape from the <Run> mode.

viii. Pour approximately 50 ml of Rhotamine WT working standard into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip and gently shake the sonde so-as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.

ix. Fill the calibration cup with at least 180 ml of fresh Rhotamine WT working standard and set it on the bench below the sonde. Do not attach it to the sonde body.

x. Go to the <Run> menu and activate <Discrete Sample> mode with a 4-second sample interval.

xi. Actuate the wipers to remove any air bubbles from the face of the chlorophyll probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

xii. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde and dye standard are not all at the same laboratory temperature). Once the chlorophyll reading has stabilized, record it in the calibration log along with the temperature of the dye standard.

xiii. Escape from the <Run> mode.

xiv. Use the temperature compensation chart to determine the appropriate chlorophyll equivalent of the dye standard at this temperature from Table 2 of YSI Technical Note: Alternate Calibration Methods for the YSI 6025 Chlorophyll Sensor Appendix 5). Record this value in the calibration log.

xv. Rinse probes with DI water and pat dry with Kimwipes®.

xvi. Pour approximately 50 ml of turbidity standard (used is acceptable) into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip and gently shake the sonde so-as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.

xvii. Fill the calibration cup with at least 180 ml of fresh turbidity standard and set it on the bench below the sonde. Do not attach it to the sonde body.

xviii. Actuate the wipers to remove any air bubbles from the face of the turbidity probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

xix. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde and turbidity standard are not all at the same laboratory temperature). Once the turbidity reading has stabilized, record it in the calibration log.

xx. Escape from the <Run> mode.

xxi. Rinse probes with deionized water, put approximately 0.5 cm of tap water in the bottom of the calibration cup and firmly attach it to the sonde.

2.4.8.1.8 LiCor 192SA PAR sensor calibration check

The LiCor PAR sensors also need to be checked for drift. Although biofouling should not be a problem with the YSI-integrated PAR sensors (with wiper brushes), the post calibration check should be done with any biofouling still in place. Repeat the steps from the initial calibration check (on page 64) and record results in the calibration log. After the post-calibration, clean the PAR sensors with water and a soft sponge or brush. Be careful not to scratch the acrylic diffuser or damage the black paint within the annular ring on the face of the sensor. Do not use alcohol, organic solvents, abrasives, or strong detergents to clean the diffuser element. Mild dishwashing detergent and vinegar are acceptable cleaning agents.

2.4.9 *Data Upload*

After completing the post-deployment calibration check, immediately upload data from the sonde to computer. Connect the sonde to your computer using the dry calibration cable, launch YSI EcoWatch software, and establish communication with the sonde. From the sonde's <File> menu, <Upload> the data file to computer using the PC6000 format. This format results in a computer file with a .dat file extension. The default program for this extension will be YSI EcoWatch, and the file will not be readily editable (making it ideal for archiving purposes). Use EcoWatch to open the date file and examine it to ensure it has not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data file and confirm that it too is

readable using EcoWatch. After these steps have been completed, reestablish communication with the sonde, select the <File> menu and <Delete all files>.

2.4.10 *Probe Care and Storage*

Of the probes used for this protocol, the YSI 5662 Dissolved Oxygen Probe has the most limited life expectancy. Under normal circumstances, it can be expected to perform well for at least 2-3 years, and will require resurfacing during this period. Consequently, it is advisable to keep replacement probes on hand to replace a probe that fails to calibrate properly. Note, however, that the dissolved oxygen probe also has a limited shelf life, so replacements should not be purchased too far in advance.

For short term storage of probes (one month or less), it is important to keep them in a humid environment while not immersing them in water. Simply keep the probes attached to the sonde, place approximately 1 cm of tap water in the calibration cup, and attach it tightly to the sonde.

Because this protocol does not call for a pH probe, long term storage of the sonde is simplified from the standard method described in the YSI Operations Manual. Completely fill the calibration cup with tap water and attach it to the sonde body. Remove the batteries from the battery chamber and replace the battery lid following the methods detailed in the YSI Operations Manual.

2.5 SOP 5 – Spatial Water Quality Monitoring with the YSI Sonde

2.5.1 *Background and Familiarization with Instruments*

This SOP describes operation of the YSI 6600 multi-parameter water quality monitor (sonde) for spatial surveys of water quality parameters at stations throughout each of the NCBN parks during a four-week late summer index period. Data are collected using the YSI 6600 deployed in discrete sampling mode, and logged to the YSI650MDS. The SOP describes a standard procedure on how to prepare, calibrate, program and upload data from these instruments. It also provides detailed methods for operating the instrument in the field. The sonde chosen for continuous monitoring is the YSI 6600EDS (Extended Deployment System) with additional modifications to accept input from two LiCor PAR sensors. The 6600EDS model may also be used for the spatial survey; however, the EDS wiper and PAR input capabilities are not needed for this SOP. The advantage of using the 6600EDS is that sondes can then be swapped between tasks for spatial and continuous monitoring. The disadvantage is the additional cost of the EDS and PAR capabilities, which are not required for this SOP. Both sondes calibrate identically. Whichever sonde is selected shall be connected to a YSI 650MDS Multiparameter Display System using a field cable of appropriate length for the individual park.

It is outside the scope of this SOP to review all of the information and methods that are required to operate the YSI 6600 or 6600EDS sonde and the 650MDS and to make effective and accurate measurements. It is imperative that both field and laboratory personnel familiarize themselves with the entire YSI 6-Series Environmental Monitoring Systems Operations Manual, the Multiparameter Display System Operations Manual, and the Technical Notes and Technical Documents available from YSI at their web site and attached herein. The purpose of this SOP is to standardize instrument handling, maintenance, calibration, and use for all the NCBN parks. It provides step-by-step instructions as they pertain specifically to this monitoring protocol; however, it does not alone provide enough guidance to ensure consistent and accurate measurements. YSI, Inc offers coursed on instrument calibration and maintenance. We strongly advise that all NACP personnel involved in instrument calibration receive annual training from YSI.

YSI, Inc. offers three different depth models of the 6600 (or 6600EDS): shallow (9 m), medium (61 m), and deep (200 m). Although all three models have the same resolution (0.001 m), the accuracy of the shallow water model (±2 cm) is considerably better than that of the medium depth model (±12 cm). The shallow model should be selected for parks where depths are not expected to exceed 9 m; the medium depth unit should be selected for the remaining parks. In either case, the sonde should be non-vented for pressure, and equipped with the following sensors:

- YSI 5662 Dissolved Oxygen Probe
- YSI 6136 Turbidity Probe
- YSI 6025 Chlorophyll Probe
- YSI 6560 Conductivity & Temperature Probe

Note that a pH sensor is not included in the above list. The 6600 and 6600EDS are routinely shipped with a pH sensor, but it should be removed from the order for a purchase credit.

YSI, Inc. also offers several options on the 650MDS. This SOP calls for the "650-04" configuration which includes the 1.5MB high-memory option and an integrated barometer. The rechargeable battery pack is not recommended. In addition to the sonde, sensors, and display system described above, this SOP calls for the following equipment and supplies:

- personal computer with an RS232 compliant serial port (or USB to serial converter) and running the current version of YSI EcoWatch software
- YSI 6-Series Environmental Monitoring Systems Operations Manual, and YSI Technical Notes and Technical Documents attached herein and also at: <http://www.ysi.com/extranet/EPGKL.nsf/SearchDocs/1F24980CDDEE02AC85256CEF00763EC1!OpenDocument>.
- YSI 650 MDS Multiparameter display System Operations Manual
- YSI 6067B Dry Calibration Cable
- YSI 651174 PC Interface Cable.
- YSI 5775 Oxygen Probe Service Kit*
- YSI 6144 Wiper Pad Kit*
- Thermometer – NBS traceable with a tolerance of ± 0.2 ˚C
- C-size alkaline batteries: 8 for the 6600 sonde and 4 for the 650MDS (do not substitute rechargeable batteries or battery packs)
- Deionized water (ASTM type III , Laboratory Grade, or better)
- 10 mS/cm conductivity standard (YSI # 3163 or equivalent)
- Turbidity standard of AMCO-AEPA-1® Microspheres (YSI # 6073)
- 1 Large ring stand or other home-made bracket that will allow for the 6600EDS to be suspended from its bail and hang so that the face of the turbidity sensor will be no less than 7.6 cm (3 in) from the bottom of the calibration cup sitting on the bench beneath it.
- Laboratory glassware for preparation and dilution of standards
- Field Cable: YSI 6091-25 ft (for shallow model sonde) or 6092-50 ft (for medium depth sonde)
- YSI 6115 GPS Cable
- GPS unit: WAAS compliant with output capabilities using the NMEA 0183 protocol
- If the GPS output is not from a DE-9 connector (9-pin D-Subminiature) you will also need a DE-9 adaptor
- Modified 5-gal plastic bucket for transporting sonde
- Calibration Logging Form (Appendix 4)

* These are expendable items provided in limited quantity with the original purchase of YSI 6600EDS sondes.

2.5.2 *Pre-Deployment Preparation and Calibration Methods*

2.5.2.1 *Preparation and programming of the YSI 650MDS data logger & display*

The YSI 650MDS serves several functions. It provides a user interface to the sonde while in the field, it logs data from the sonde during hydrocasts, and it provides a real-time display of the output from each of the sonde sensors. At the beginning of each field season, install four fresh C-size alkaline batteries according to the directions in the 650MDS Operations Manual. Pay particular attention to the installation of the battery cover O-ring. These batteries are likely to last for the duration of the index period, but you should monitor their charge level (see thermometer bar at bottom right corner of display) and replace them as needed. Note that should the sonde batteries get low, it is possible to power the sonde from the batteries in the 650MDS ("power sonde" option under the <System Setup> menu). It is also prudent to bring a spare set of batteries into the field.

Before the instrument can be deployed, it must be properly programmed. There are three menu sections that you will need to program: <File>, <Logging Setup> and <System Setup>.

2.5.2.1.1 650MDS <File> menu

Check to confirm that there are no remaining data (.dat) files stored in the 650MDS memory. All files should previously have been removed as part of the SOP for downloading data to computer, so the presence of any data files will indicate a failure to follow the SOP. Proceed with caution! Download any files to a specially labeled directory and annotate the calibration log to this effect. Use YSI EcoWatch software to open the date files and examine them to ensure they have not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data files and confirm that they too are readable using EcoWatch. After these steps have been completed, select <Delete all files> from the 650MDS <File> menu.

2.5.2.1.2 650MDS <Logging Setup> menu

From the main menu in the 650MDS, go to <Logging Setup> and program as follows:

i. Set the sampling interval to one second (= 00:00:01)

ii. Enable "Use site list"

iii. Enable "Store Barometer"

iv. Enable "Store Lat and Long"

v. Enable "Store site number"

vi. Select <Edit Site List> and create a new file for each of the 30 spatial elements of the sampling design.

   a. As a standardized naming convention, each file name should start with the 4-character park code followed by a hyphen and the two-numeral spatial element number (e.g. COLO-Ø1).

b. If there should be the need for more than one file per single spatial element (more than one station sampled per element), add a letter code as the 8th character in the file name, and annotate your field notes accordingly (e.g. COLO- Ø1A).

c. For discrete measurements made at the continuous logging station, substitute "LS" for the spatial element number followed by a number that MUST increment each time the continuous logging station is visited during the field season (e.g. COLO-LS1).

d. In the "Site Number" field for each site, enter the spatial element number. Use Ø for the logging station.

e. The "Site Name" field is for your convenience only. It does not get appended to the data file, but you may find it useful for differentiating between multiple stations within one spatial element (as in example –b above) and for keeping track of dates associated with each unique file name for the logging station (as in example –c above).

2.5.2.1.3 650MDS <System Setup> menu

From the main menu in the 650MDS, go to <System Setup> and program as follows:

i. Set the correct date and LOCAL TIME. For all anticipated use of the SOP, this will be Eastern Daylight Savings Time. Select the "4 digit year" option.

ii. Make certain that the "Power sonde" and ""Comma radix" options are NOT selected.

iii. Set the "Shut off time" to 30 minutes.

This menu also provides for user calibration of the barometer. It is recommended that the barometer be checked annually for drift. Recalibration is a delicate procedure. Carefully follow the guidance in the 650MDS Operations Manual and be certain that you are calibrating against barometric pressure that has not been corrected for altitude (the National Weather Service generally reports barometric pressure corrected to sea level). If in doubt, return the unit to YSI for a factory recalibration.

2.5.2.2 *Preparation and calibration of the YSI 6600 (6600EDS) sonde*

2.5.2.2.1 General notes

During the calibration procedure, never accept any calibrations that have produced a warning message. You must instead determine the cause of the problem, correct it, and recalibrate following this SOP and the instrument manual.

Each field season, batteries, dissolved oxygen membranes, and wiper pads for the turbidity and chlorophyll probes must all be replaced with new ones. Pay particular attention to YSI guidance on appropriate clearances for wiper assemblies since excessively tight installation could slow down or stall the motor on the turbidity and chlorophyll sensors. All of these components should last for the duration of the index period, except perhaps for the dissolved oxygen membrane which is easily damaged.

Since dissolved oxygen membranes are unstable for the first 3 to 6 hours after they are installed, it is important to check their condition and performance in advance of any planned field work. This SOP calls for the DO membrane to be examined (and replaced if necessary) and the sonde calibrated 6-12 hours before deployment. After this stabilization period, just the DO sensor is recalibrated, and the sonde is ready for deployment.

Except where explicitly noted in the SOP, standards should NOT be used for more than one calibration. They may, however, be saved and used to pre-rinse the sensors and the calibration cup prior to putting them into fresh standard solutions. Rather than measure calibration solutions with graduated cylinders each time they are dispensed, you may choose to mark the outside of the calibration cup at the appropriate levels for each probe. Dispensing standards directly into the calibration cup will also help prevent contamination from dirty glassware. For many of the calibration steps, reaching a stable calibration point requires the probe and calibration solution to thermally equilibrate. This occurs more quickly if both the sonde and all the calibration solutions are at laboratory temperature. Be sure to prepare deionized water in advance and store an ample supply in a carboy.

The 6600 or 6600EDS should be calibrated while hanging from a ring stand or custom-mounted hook. Height is critical for proper calibration of the turbidity and chlorophyll sensors. The bottom face of the 6136 Turbidity Probe should be set so there is at least 7.6 cm of clearance between it and the inside bottom of the calibration cup (when unthreaded from the sonde body). This is the minimum clearance in order to avoid backscatter interference when calibrating the turbidity and chlorophyll sensors. Greater clearances will result in increased consumption of standard solutions. The conductivity probe can, however, be calibrated upside down in order to conserve calibration solution. If you are calibrating both YSI 6600 and 6600EDS sondes, note that the sonde bodies are different and require different hanging brackets or heights.

The wiper assemblies on both the turbidity and chlorophyll probes must "park" 180^{o} away from the optical windows on these probes. Actuating the wipers while in air can sometimes cause them to park in different locations. At several points in the calibration procedure you are prompted to confirm that the wipers are parking properly. If they fail to do so, troubleshoot using guidance in the YSI 6-Series Operations Manual.

Taking the above notes into account, the following calibration procedure should be completed in the order it is presented.

2.5.2.2.2 Instrument Preparation

Before the instrument can be deployed, it must be calibrated and several maintenance steps performed. Unlike for continuous monitoring, it is not necessary to paint the bulkhead, probe guard, and individual sensors with marine ablative antifouling paint. Assemble the individual sensors onto the sonde per instructions in the manual using extra care not to cross-thread the sensors when installing them onto the softer PVC sonde bulkhead. As a final check prior to calibration, visually inspect the sonde for any abnormalities.

Install eight fresh C-size alkaline batteries according to the directions in the YSI Operations Manual. Pay particular attention to the installation of the bottom O-ring, as this is the one that keeps the battery compartment from leaking. It must be properly seated in its groove on the bottom of the battery lid, clean and free from contaminants, and lubricated with a VERY light film of clean silicone grease. The O-ring on the side of the battery lid seals out contaminants and fouling material and should also be cleaned and lubricated with a light film of clean silicone grease. Also note that over tightening the battery lid screws may distort the lid and cause the battery compartment to flood.

2.5.2.2.3 Standard solutions

Calibration standards used for this SOP are identical to those used for calibration of the YSI 6600EDS for continuous monitoring. Refer to section on "Standard Solutions" in that SOP for details on deionized water, turbidity, and Rhotamine WT dye standards.

The only difference between the SOPs is that a distinction is needed in the rationale for calibrating the chlorophyll sensor with Rhotamine WT dye. For continuous monitoring, the primary role of calibrating the chlorophyll sensor with Rhotamine WT dye is to evaluate sensor drift over time. For spatial survey monitoring, sensor drift is not an issue, but dye calibration is still included in the SOP. For both SOPs, the primary method for calibrating the chlorophyll sensor (fluorometer) involves creating a standard curve between values reported by the sensor and independent values of chlorophyll concentration determined by extractive laboratory methods from field grab samples (see SOP on Chlorophyll Methods). Should there be a problem with these discrete chlorophyll samples, however, the dye calibration will provide at least some information on relative chlorophyll concentrations. Over time, it should also allow the NCBN to develop park-specific empirical relationships between the responses of the chlorophyll sensor to Rhotamine WT dye and to natural phytoplankton populations.

2.5.2.2.4 Dissolved Oxygen

ix. Inspect the DO probe anodes. If they are darkened or gray in color, recondition using the 6035 reconditioning kit.

x. Inspect the DO membrane. It should be undamaged, tightly stretched, wrinkle free, and there should be no air or gas bubbles under it. If necessary, install a new membrane following guidance in the YSI 6-Series Operations Manual and Technical Notes.

xi. Hang the sonde from the ring stand or custom support bracket so that the turbidity probe is on the left, and the chlorophyll probe on the right. Put approximately 0.5 cm of water in the bottom of the calibration cup and loosely attach it to the sonde (engaging several threads). Attach the Dry Calibration Cable between the sonde and computer, launch EcoWatch software, and establish communication with the sonde.

xii. Go to the sonde <Report> menu and enable the <DO Charge> option.

xiii. Go to the sonde <Advanced> <Setup> menu and disable the "RS-232 auto-sleep" function.

xiv. If you have just installed a new membrane, go to the "<Run> menu and start the sonde in the <Discrete Sample> mode with a 4-second sample interval. Allow the sonde to run for 10 minutes to "burn in" the new membrane.

xv. Perform a High-to-Low Transmission Test: Start the sonde in <Discrete Sample> mode with a 4 second sample interval. Disregard the first two DO % Saturation samples, and then record the next 10 samples. The DO % Saturation values must start high and drop with each 4-second reading. It does not matter if the readings do not reach 100%, but there must be a high to low trend. If the recorded values start low then climb upward, the sensor has a problem and must be reconditioned or replaced. Record the 10 readings and the pass-fail status of the test on the calibration log. Escape from the <Run> mode.

xvi. Wait at least 5 minutes in the idle (not <Run>) mode before proceeding. You must also wait at least 10 minutes from the time the calibration cup (with 0.5 cm water in bottom) was attached to the sonde body. Once these minimum times have been reached, calibrate the DO sensor using the <% Saturation> method. Be sure to check and enter the current barometric pressure. Note that laboratory barometer readings are usually "true" values of air pressure and can be used "as is" for oxygen calibration. The barometer on the 650MDS (if properly calibrated) also reports "true" barometric pressure and can be used "as is." Pressure reported by the National Weather Service, however, is usually "corrected" to sea level and cannot be used in raw form. See the YSI Operations Manual for correction methods. Record the DO Charge on the calibration log. It should be 50 ± 25.

xvii. When the calibration has been accepted, check the "DO gain" for the sensor and record it on the calibration log. This is found under the sonde's <Advanced Menu> under the <Cal Constants> option. The target value for gain is 1.0, with an acceptable range from 0.7 to 1.4.

2.5.2.2.5 Conductivity

ix. You will need approximately 425 ml of conductivity standard to calibrate this probe. This volume can be reduced to approximately 225 ml if the sonde is held upside down while performing the calibration.

x. Calibration of the conductivity probe is very vulnerable to contamination. Before performing the calibration, the calibration cup and all the surfaces of all the probes must be triple rinsed with a small amount of conductivity standard. Used standard from previous execution of this SOP is acceptable.

xi. Pour approximately 50 ml of 10 mS/cm conductivity standard into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip and gently shake the sonde so-as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution (do not re-use rinse standards). Repeat this step two more times.

xii. Pour 425 ml of FRESH 10 mS/cm conductivity standard into the calibration cup and attach it to the sonde body (225 ml if calibrating the sonde upside down).

xiii. Insure that the conductivity probe is completely submerged in the standard. The hole in the side of the probe must be under the surface of the solution and not have any trapped bubbles in the opening.

xiv. Initiate calibration of the probe selecting the <specific conductance> option and entering "10" for the conductivity of the standard (Be sure to enter the conductivity of the standard in mS/cm, not µS/cm).

xv. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde, the conductivity standard and the deionized water from previous steps are not all at the same laboratory temperature). After no changes occur in the reading for approximately 30 seconds, accept the calibration (hit <enter>) and record on the calibration log the last value reported prior to pressing <enter>. With an NBS-traceable thermometer, verify the accuracy of the sonde temperature reading and record it on the calibration log.

xvi. When the calibration has been accepted, check the "Conductivity Cell Constant" for the sensor and record it on the calibration log. This is found under the sonde's <Advanced Menu> under the <Cal Constants> option. The target value for this probe is 5.0 ± 0.45. Numbers outside this range usually indicate a problem in the calibration process or a contaminated standard. Never override a calibration error message and investigate any "Out of Range" report.

2.5.2.2.6 Chlorophyll

xvii. You will need deionized water and a working concentration of Rhodamine WT dye standard to calibrate the chlorophyll fluorometer. Prepare the working Rhodamine standard following the procedures in section on Standard Solutions in the SOP for Continuous Water Quality Monitoring.

xviii. Half fill the calibration cup with deionized water and attach it to the sonde body. Swirl, tip and gently shake the sonde in order to thoroughly wet all of the probe and bulkhead surfaces. Discard this rinse water and repeat two more times.

xix. Fill the calibration cup with at least 180 ml of fresh deionized water and set it on the bench below the sonde. Do not attach it to the sonde body. The distance between the face of the turbidity probe and the inside bottom of the calibration cup should be no less than 7.6 cm.

xx. Select the <2 point > method from chlorophyll calibration menu, and enter zero <0> as the value for the first point. Start the calibration routine and actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

xxi. If the deionized water is already at laboratory temperature wait approximately 1 minute for thermal equilibration. Once a stable reading has been maintained for approximately 30 seconds, press <enter> to set the calibration. On the calibration log, record the last value displayed before pressing <enter>. Note: chlorophyll values tend to bounce more than other values, this is normal.

xxii. Pour approximately 50 ml of Rhotamine WT working standard into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip and gently shake the

sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.

xxiii. Fill the calibration cup with at least 180 ml of fresh Rhotamine WT working standard and set it on the bench below the sonde. Do not attach it to the sonde body.

xxiv. Confirm that the standard is at laboratory temperature, then use the temperature compensation chart to select the proper chlorophyll concentration to enter as the second point in the calibration from Table 2 of YSI Technical Note: Alternate Calibration Methods for the YSI 6025 Chlorophyll Sensor (Appendix 5).

xxv. Continue the calibration routine and actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

xxvi. Allow the sonde to run until a stable reading is maintained for approximately 30 seconds, then press <enter> to set the calibration. On the calibration log, record the last value displayed before pressing <enter>. Rinse probes with DI water and pat dry with Kimwipes®.

xxvii. When the calibration has been accepted, check the "Chlorophyll Offset" for the sensor and record it on the calibration log. This is found under the sonde's <Advanced Menu> under the <Cal Constants> option.

2.5.2.2.7 Turbidity

vi. The sonde and calibration cup must be clean or contamination of the zero NTU standard will occur. Half fill the calibration cup with deionized water and attach it to the sonde body. Swirl, tip and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this discard this rinse water and repeat two more times.

vii. Fill the calibration cup with at least 180 ml of fresh deionized water and set it on the bench below the sonde. Do not attach it to the sonde body. The distance between the face of the turbidity probe and the inside bottom of the calibration cup should be no less than 7.6 cm.

viii. Select the <2 point > method from turbidity calibration menu, and enter zero <0> as the value for the first point. Start the calibration routine and actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

ix. Wait for a stable reading to be maintained for approximately 30 seconds, then press <enter> to set the calibration. The reading on-screen should now be approximately 0.0. On the calibration log, record the last value displayed before pressing <enter>.

x. Pour approximately 50 ml of turbidity standard (used is acceptable) into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.

xxviii. Fill the calibration cup with at least 180 ml of fresh turbidity standard and set it on the bench below the sonde. Do not attach it to the sonde body.

xxix. Continue the calibration routine entering 123 NTU for the second standard. (NOTE: the recommended AMCO-AEPA-1® standard has a different value depending upon the sensor. It is 123 NTU for the YSI 6136.) Actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180º away from the optical window on the probe face.

xxx. Allow the sonde to run until a stable reading is maintained for approximately 30 seconds, then press <enter> to set the calibration. On the calibration log, record the last value displayed before pressing <enter>.

xxxi. When the calibration has been accepted, check the "Turbidity Offset" for the sensor and record it on the calibration log. This is found under the sonde's <Advanced Menu> under the <Cal Constants> option.

xxxii. Rinse probes with deionized water, put approximately 0.5 cm of water in the bottom of the calibration cup and firmly attach it to the sonde (sealed, not loosely threaded).

2.5.2.2.8 Pressure/Depth calibration

iv. From the <Calibrate> menu, select <Pressure-Abs>.

v. Input 0.00 (or a know sensor offset in feet if working far from sea level).

vi. Wait until no significant change occurs for approximately 30 seconds, then press <enter> to confirm the calibration.

2.5.2.2.9 Sonde programming and miscellaneous preparations

When preparing the sonde for discrete sampling, realize that all data will be logged directly to the YSI 650MDS data logger and display. Methods for programming the 650MDS are given elsewhere in this SOP (section 2.5.2.1 on page 84). Program the 6600 or 6600EDS sonde with the correct date and LOCAL TIME. For all anticipated use of this SOP, this will be Eastern Daylight Savings Time. The following report parameters should be enabled in the <Report> menu: date, time, temperature, specific conductance, salinity, dissolved oxygen saturation, dissolved oxygen concentration, DO charge, depth, turbidity, chlorophyll, and battery voltage. Set the sampling interval to 1 second. This option is found under the <Run> <Discrete Sample> menu.

Finally, check to confirm that there are no remaining data (.dat) files stored in the sonde's memory. If this sonde was previously used for continuous monitoring, all files should previously have been removed as part of the SOP for downloading data to computer. Since this SOP calls for data to be logged to the 650MDS, the presence of any data files in the sonde's memory will indicate a failure to follow the SOPs of this or other protocols. Proceed with caution! Download any files to a specially labeled directory and annotate the calibration log to this effect. Use YSI EcoWatch software to open the date files and examine them to ensure they have not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data files and confirm

that they too are readable using EcoWatch. After these steps have been completed, select <Delete all files> from the sonde's <File> menu.

2.5.2.2.10 Final lab calibration of dissolved oxygen and preparing the sonde for field work

i. Just prior to bringing the sonde into the field for deployment, perform a final lab calibration of the dissolved oxygen probe. To do this, go to the <calibrate> menu and repeat the last 2 steps from section 2.5.2.2.4 on page 87.

ii. Terminate communication with the computer, disconnect the dry calibration cable, and install the field cable. Attach the strain relieve connector between the field cable and the bail on the sonde.

iii. Attach the other end of the field cable to the YSI-6115 GPS cable, and the other end of the GPS cable to the 650MDS. Attach the strain relieve connector between the field cable and strain relief lanyard on the 650MDS.

iv. Attach the GPS unit to the YSI-6115 GPS cable (using a DE-9 adaptor if needed) and temporarily power up the 650MDS and GPS to confirm communication between them. Make certain that you have satellite reception (may be a problem indoors) and look for latitude and longitude to be displayed in the bottom left corner of the 6550MDS screen.

v. Remove the calibration cup from the sonde body and install the sensor guard. It is best not to attempt this in the field since the oxygen membrane is vulnerable to being damaged if it bumped by the guard.

vi. Wrap the sonde in a white towel that has been soaked in tap water. The towel should cover the entire sensor guard and go around it at least twice. This will provide a humid environment for the sensors, protection from thermal extremes, and some degree of shock protection.

vii. Transport the sonde to the field in a specially-modified white 5-gallon plastic bucket. To prepare the bucket, place approximately 8 kg of lead weight in its bottom. Neoprene-covered soft weight packs containing lead shot that are used by SCUBA divers are excellent because they will not risk damaging the sonde. Also put approximately 2 cm of water in the bottom of the bucket. Cut a hole in the middle of the lid that will accommodate the sonde body wrapped in the towel. The lid will support the sonde, help to maintain the humid environment, and keep water from spilling or sloshing out.

viii. As much as possible, keep the sonde & bucket out of direct sunlight to prevent thermal extremes.

2.5.3 *Making Measurements*

2.5.3.1 *Standard Approach*

i. The sonde must be allowed to warm up for 4 to 5 minutes before taking measurements. Turn on the sonde by powering up the 650MDS and selecting <Sonde run> from the main menu.

ii. Once the sonde has warmed up, and with the unit still wrapped in the wet towel inside the transportation bucket, check the dissolved oxygen percent saturation ("DO%") and record this value on the Spatial Survey Station Data Sheet.

iii. Confirm that it reads 100% ± 2%. **If** the dissolved oxygen percent saturation has drifted beyond these tolerances, the dissolved oxygen channel must be recalibrated on the spot. This is accomplished as follows:

a. Press <Escape> to get to the 650 MDS Main Menu

b. select <Sonde Menu>

c. select <Calibrate>

d. select <Dissolved Oxy>

e. select <DO%>

f. update the barometric pressure used for the calibration (large font numerals) so that it exactly matches the barometric pressure as measured by the 650MDS (small font numerals in the bottom right corner of the screen).

g. press <enter> and wait for the sensor to stabilize and the sensor to calibrate. Should the sensor fail to calibrate, follow trouble-shooting guidance in the YSI 6-Series Environmental Monitoring Systems Operations Manual.

h. Press <Esc> to escape back to the 650MDS main menu, and then select <Sonde Run>.

i. Confirm that the dissolved oxygen percent saturation ("DO%") reads 100% ± 2% and check off the box on the Spatial Survey Station Data Sheet that the sensor was successfully recalibrated

iv. With the sonde still wrapped in the wet towel inside the transportation bucket, select <start logging> from the "650" side of the menu (not the "sonde" side). This will log the data to the 650MDS rather than the sonde. Logging is started before putting the sonde into the water so that the pre-calibration data are stored right in the site data file along with the actual profile data.

v. Select the appropriate <Filename> into which the data will be stored (see section 2.5.2.1.2) and confirm that the 650MDS displays "650 is Logging" at the top left of the screen.

vi. Lower the sonde over the side, submerging it in the water, and wait for the temperature to equilibrate. The length of time will depend upon the temperature differential between the air and water. Watch for the temperature readout to stabilize. While waiting, select <clean optics> from the "sonde" side of the menu.

vii. Once the optics have finished being wiped and the temperature has stabilized, raise the sonde so that the pressure ports are just out of the water, but the entire sensor guard is still underwater.

viii. Slowly lower the sonde through the water column at the rate of approximately 10-20 cms^{-1}. Lower to within 50 cm of the bottom, then retrieve the sonde at approximately the same rate. Avoid running the sonde into the bottom since hydrogen sulfide will interfere with the Clarke-type electrode used for dissolved oxygen and make the output jumpy. This effect is also seen when the bottom water is anoxic and H_2S is present.

ix. At the surface, select <stop logging> from the "650" side of the menu, wrap the sonde in the wet towel, and return it to the protective bucket.

x. If the interval of time before the next warm-up is expected to be less than 5 minutes, then it is appropriate to leave the sonde running between stations. Otherwise, the sonde should be powered off to conserve battery power.

2.5.3.2 *Accommodation for deeps systems*

Somes Sound at Acadia National Park is over 45 m deep in some locations. The medium-depth YSI 6000-series sonde is capable of measuring to these depths, but the longest stock field cable is only 30m (100ft) long. Beyond this length, hand-lowering of the sonde becomes logistically impractical and potentially unsafe. The recommended solution for these situations is to reprogram the sonde to collect data in autonomous mode, then to lower it on a mechanically or hydraulically powered wire. Extra ballast shall be attached to the wire to assist in keeping a vertical wire angle. Since there will be no display on which to track the sonde's depth, extra precautions must be taken not to run the sonde into the bottom. The research vessel will not be able to anchor for these stations, so vessel drift must also be taken into account in order to avoid dragging the sonde up onto shallower bottom. Program and deploy the sonde for these stations as follows.

i. Complete steps i-iii from section 2.5.3.1.

ii. Select <Run> from the sonde main menu and initiate <unattended sample> mode. The next window will prompt you to enter the sampling interval (one second or 00:00:01), the start date (enter today's date), and the start time. Enter the current time plus four minutes. This will give you time to deploy the sonde before the wipers activate and also allow the sonde to thermally equilibrate. Keep the sampling duration at the default (365 days). Name the file to which you will log using the spatial survey naming convention (see section 650MDS <Logging Setup> menu on page 84).

iii. Select start logging, and start timing the four-minute delay. Disconnect the field cable from the sonde and replace it with a bulkhead connector cap.

iv. Deploy the sonde over the rail, suspending it from the wire so that sonde is submerged. Wait (using a timer) for the wipers to activate.

v. While waiting, record the current barometric pressure and position (Lat and Long.) on the Spatial Survey Data Sheet. Both of these are shown at the bottom of the 650MDS display screen.

vi. Once the wipers have finished, raise the sonde until the pressure port is just underwater, the begin lowering the sonde through the water column. If it isn't possible

vii. Use wire-out readings and wire angle to estimate depth of the sonde. This will provide only a approximation of depth, so the sonde should be lowered no closer than 3 m off the bottom.

viii. Raise the sonde back to the surface, reconnect the 650MDS, and connect to the sonde Main Menu. Select <run>, then <unattended sample>, then "stop logging."

ix. Record the GPS position for the end of the hydrocast on the Spatial Survey Data Sheet.

2.5.4 *Data Upload*

Upload data from the 650MDS to computer at the end of each field day. Connect the 650MDS to your computer using the YSI 655174 PC interface cable, launch YSI EcoWatch software, and establish communication with the 650MDS. From the <File> menu, <Upload> all the data files collected that day using the PC6000 format. This format results in a computer file with a .dat file extension. The default program for this extension will be YSI EcoWatch, and the file will not be readily editable (making it ideal for archiving purposes). Note that file names entered as part of the "Site List" are not created until data is logged into them. Only stations where data was collected will have data files.

Use EcoWatch to open each date file that you just downloaded and examine them to ensure they have not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data files and confirm that they too are readable using EcoWatch. After these steps have been completed, reestablish communication with the 650MDS, select the <File> menu and <Delete all files>.

If survey data was logged into sonde memory rather than to the 650MDS, then follow guidance from the SOP on Continuous Water Quality Monitoring with the YSI Sonde to upload these data. This will apply only to stations that are deeper than the available stock field cable (Acadia Somes Sound only).

2.5.5 *Probe Care and Storage*

Of the probes used for this protocol, the YSI 5662 Dissolved Oxygen Probe has the most limited life expectancy. Under normal circumstances, it can be expected to perform well for at least 2-3 years, and will require resurfacing during this period. Consequently, it is advisable to keep replacement probes on hand to replace a probe that fails to calibrate properly. Note, however, that the dissolved oxygen probe also has a limited shelf life, so replacements should not be purchased too far in advance.

For short term storage of probes (one month or less), it is important to keep them in a humid environment while not immersing them in water. Simply keep the probes attached to the sonde, place approximately 1 cm of tap water in the calibration cup, and attach it tightly to the sonde.

Because this protocol does not call for a pH probe, long term storage of the sonde is simplified from the standard method described in the YSI Operations Manual. Completely fill the calibration cup with tap water and attach it to the sonde body. Remove the batteries from the battery chamber and replace the battery lid following the methods detailed in the YSI Operations Manual.

2.6 SOP 6 – Spatial Water Quality Monitoring with LiCor PAR Instruments

2.6.1 *Introduction*

This SOP describes the methods for spatial monitoring of underwater photosynthetically available radiation (PAR) throughout the NACP. At each park, 30 stations are occupied during a four-week late summer index period. Depth profiles of PAR are measured using a LiCor LI-1400 console equipped with one underwater quantum sensor (LI-192SA) and one quantum sensor in air (LI-190SA "deck sensor"). The attenuation coefficient of downwelling PAR (K_d) is calculated from these data. Caruthers et al. (2001) provide a good primer on measuring the attenuation coefficient of PAR. We specify 2-pi sensors because they are robust and easier than 4-pi sensors to keep free of biofouling over long-term deployments. Although not relevant to this SOP, it makes practical sense to use the same sensor design for both spatial and continuous monitoring. Although 2-pi sensors theoretically measure downwelling rather than scalar irradiance (4-pi) the difference in attenuation coefficient measured with the different sensors is extremely small and negligible (Gallegos 1993, Moore and Goodman 1993, Moore et al. 1997).

Photosythetic photon flux density (PPFD) is a highly erratic variable, so a certain degree of time averaging is necessary in order to smooth the data. This SOP calls for 15 second averaging of irradiance at each depth in the profile. It also calls for irradiance at depth to be reported as a percentage of the instantaneous irradiance in air. This latter step corrects for any error that would be introduced by changes in the incident solar irradiance over the period of time it takes to complete a full profile of the water column.

The purpose of this SOP is to standardize instrument programming, assembly, calibration, and use throughout the NACP. It provides step-by-step instructions as they pertain specifically to this monitoring protocol. It is outside the scope of this SOP to comprehensively review all of the information and methods that are required to operate these LiCor instruments and to make effective and accurate measurements. It is imperative that both field and laboratory personnel familiarize themselves with the LiCor LI-1400 Datalogger Instruction Manual and the LiCor Radiation Sensors Instruction Manual (for both the terrestrial and underwater type SA sensors). Although the LI-1400 has data logging capabilities, data are recorded manually in this protocol. The LI-1400 serves here as an easily programmable controller and display interface for the PAR sensors and performs some simple calculations and data averaging functions. It is possible to use the logging function as well, but the nature of the data from this SOP make manual logging more expedient.

Equipment requirements for this SOP include the following:

- LiCor LI-1400 data logger
- LiCor LI-192SA Underwater Quantum Sensors (2-*Pi* cosine-corrected)
- LiCor LI-190SA Terrestrial Quantum Sensors (it is recommended that this NOT be the sensor reserved for calibration purposes in the SOP on Continuous Monitoring with the YSI sonde)
- LiCor LI-2222UWB 10 m underwater cable

- LiCor LI-2003S mounting and leveling fixture for LI-I90SA quantum sensor.
- LiCor LI-2009S lowering frame
- LiCor LI-1400 Data Logger Instruction Manual
- LiCor Radiation Sensors Instruction Manual: Terrestrial Type SA
- LiCor Radiation Sensors Instruction Manual: Underwater Type SA
- Personal computer with an RS232- compliant serial port (or USB to serial converter)
- RS232 cable (DTE to DTE with DE9 connecters)
- LiCor LI-1400-501 PC Communications Software
- AA-size alkaline batteries
- 7 oz "egg" or "bank" lead sinker

2.6.2 *PAR Sensor Calibration*

LiCor, Inc. recommends biennial factory calibration of its LI-192SA and LI-190SA quantum sensors. While more frequent factory calibration is recommended for sensors deployed by the NPS for continuous monitoring (see SOP on Continuous Water Quality Monitoring with YSI Sonde), biennial calibration is sufficient for the discrete profiling described here. Should, however, the acrylic diffuser lens become scratched or the black paint (in the annular recess around the diffuser) peel or fade, then repair and recalibration is required. Both deck (LI-190SA) and underwater (LI-192SA) PAR sensors are factory calibrated in air. "Multipliers" are provided by LiCor that allow the electrical output for individual sensors to be converted into real values of PPFD (in units of µmoles photons $m^{-1}s^{-1}$). For the underwater sensor, both "in air" and "in water" multipliers are reported by LiCor. The latter accounts for immersion effects and is calculated from the "in air" value based upon an empirical relationship ("in water"="in air"/ 1.32).

LiCor, Inc provides weatherproof calibration tags as part of their calibration services. For the deck sensor (190SA), this should be kept attached to the sensor's cable. For the underwater sensor (192SA) this should be attached to the deck end of the underwater cable used for profiling. This provides a quick reference for checking that correct multipliers have been entered into the LI-1400.

2.6.3 *LI-1400 Programming and Preparation*

At the beginning of each field season, install four fresh C-size alkaline batteries according to the directions in the LI-1400 Data Logger Instruction Manual. These batteries are likely to last for the duration of the index period. The best approach to tracking battery charge is to activate the battery voltage channel and check it regularly. As a backup to manually checking the battery voltage, the LCD display on LI-1400 will blink on and off when the voltage drops to 4.0 V (the nominal full-charge voltage is 6.0 V). The unit will then shut off when the voltage falls to 3.8 V. Batteries should be replaced well before this occurs, with the goal of avoiding the need to replace batteries in the field.

Programming the LI-1400 can be done either directly using its membrane keypad, or indirectly by downloading programming instructions from a personal computer running LI-1400-501 PC Communications Software. The latter is significantly more intuitive and is described in the following steps.

i. Launch the LI-1400-501 software and open a "NEW" programming document from the <File> menu

ii. Highlight channel <I1> and select "light" as the sensor type

iii. Fill in each of the fields as follows:

   a. <Description> - enter the serial number for the underwater sensor ("UWQxxxx")

   b. <Channel Label> - enter "PPFD" (photosynthetic photon flux density)

   c. <Multiplier> - enter the "in water" multiplier from LiCor factory calibration certificate for the underwater sensor

   d. <Average> - select 15 seconds

   e. <Logging Options> - select "none"

iv. Highlight channel <I2> and select "light" as the sensor type

v. Fill in each of the fields as follows:

   a. <Description> - enter the serial number for the deck sensor ("Qxxxxx")

   b. <Channel Label> - enter "Air"

   c. <Multiplier> - enter the multiplier from LiCor factory calibration certificate for the deck sensor

   d. <Average> - select "15 sec"

   e. <Logging Options> - select "none"

vi. Highlight channel <VB BATT VOLTAGE> and select "Battery" as the <Sensor Type> and "none" as the <Logging Routine>.

vii. Highlight channel <M1> and select "General" as the sensor type.

viii. Fill in each of the subsequent fields as follows:

   a. <Description> - enter "percent air"

   b. <Channel Label> - enter "%Air"

   c. <Input Channel> - select "I1"

   d. <Average> - select "1 sec"

   e. <Math Function> - select "none"

   f. <Channel Operation> – select "edit"

      i. <Operator> - select "/"

      ii. <Channel> - select "I2"

iii. <Function Type> – select “Poly”

iv. <Description> - leave blank

v. <Parameter a1> - enter 100

vi. leave remaining Parameters (a0 and a2-a5) set to 0

vii. select “OK”

g. <Logging Options> - select “none”

ix. Select the <File> menu and save this program as “LiCor spatial survey setup”

x. Using the RS232 cable and serial ports, establish communication between the LI-1400 and PC (select “Connect” from the <Remote> menu)

xi. Download the program to the logger (select “Send Setup” from the <Remote> menu.

xii. Reset the time on the LI-1400 to local time (select “Set Time” from the <Remote> menu. For all anticipated use of this SOP, the time standard will be Eastern Daylight Savings Time.

xiii. The LI-1400 is capable of simultaneously displaying the output from two different data channels. Follow guidance in the LI-1400 Datalogger Instruction Manual and adjust the display so that the first line is reporting output from the underwater sensor, and the second line is reporting the output from the math channel (which reports the percent of incident PAR reaching the depth of the underwater PAR sensor). If you have programmed the logger as described above, the display should look as follows (with “xxxxxx” replaced by actual values from the channels once the sensors are plugged in).

```
I1A xxxxxx UW
M1I xxxxxx %I
```

xiv. The LI-1400 is now programmed for use.

2.6.4 *Instrument Assembly*

Attach the underwater 2 pi PAR sensor to the lowering frame in the “downwelling” (upward looking) position using the insulated screws and insulating washer provided by LiCor. These provide electrical insulation to help protect against galvanic corrosion of the aluminum lowering frame. Attach the underwater cable to the PAR sensor and lowering frame follow guidance in the LiCor Radiation Sensor Instruction Manual.

Since no upwelling sensor is required for this SOP, the lowering frame will need to be balanced with weights to hang properly. Best performance and adjustability is achieved by adding approximately 210g of weight (approx 7.25 oz) in the “upwelling” sensor position. Lead “egg” or “bank” fishing sinkers work well for this purpose. This allows the lowering frame to hang properly with the least amount of weight. Additional weight can then be added to the bottom center ring as needed for different current conditions.

Attach a lowering rope to the top eye of the lowering frame. A low-stretch rope is required for this purpose. Quarter-inch double-braided polyester is preferred, but

polypropylene ropes are also acceptable (they are also low stretch but much more difficult to tie). Nylon and natural fiber ropes are unacceptable. Tie in to the lowering harness using a bowline or anchor bend. Attach the underwater cable to the lowering rope at approximately 25 cm intervals along its entire length using black plastic electricians' tape, making certain that there is slightly more slack in the cable than the rope. This will ensure that the rope is stressed rather than the cable. Using colored electricians tape, mark off depths on the lowering frame and lowering rope. Do not mark the underwater cable because your marks may migrate if the cable slips along the lowering rope. Make certain to reference all measurements against the depth of the diffuser on the PAR sensor. Depth increments should be 0.1 m for the first meter, 0.2 m for the second meter, and 0.5 m to a depth of 4 m. The rest of the cable is not needed and can be coiled and bundled with cable ties. Use an indelible marker to record the depths on the tape. Depth markings should be rechecked for accuracy at the beginning of each field season.

Install the LI-190SA terrestrial quantum sensor on the mounting and leveling fixture using the supplied Allen wrench and set screw. This deck cell will need to be placed in a location on the boat where it will not be shaded by personnel or equipment. This can be accomplished by either permanently attaching the mounting and leveling fixture to a high location on the boat (in which case the 190SA is kept in the fixture only when conducting field work), or by using heavy duty Velcro® with pressure sensitive adhesive to create several mounting locations around the vessel.

Attach the end of the underwater cable to channel I1 on the LI-1400 via the BNC connector, and attach the deck sensor to channel I2. Channels are labeled on the back of the LI-1400: when looking from the front, the underwater connector should be plugged in at the left, and the deck sensor plugged in at the center.

2.6.5 *Making Measurements*

Light profiles should be measured within three hours of local apparent noon. This is a slight extension to the interval recommended by Carruthers et al. (2001), but will still generate very good data without excessively shortening the field day. At each station, a light profile is measured by suspending the lowering frame at a series of depths and recording the underwater irradiance as both a PPFD (channel I1) and a percentage of incident PAR (channel M1). The goal is to measure these values at six to eight depths within the photic zone. Readings should be spaced closer together near the surface where PAR attenuates the fastest. A good starting point for most parks in the NACP will be to take readings at the following depths: 0.1 m, 0.2 m, 0.4 m, 0.8 m, 1.4 m, and 2.0 m. The deepest measurement should read below 5% on channel M1. Add additional depth readings up to 3.0 m until this is achieved or the bottom is reached. For very turbid and/or shallow stations, compress the six to eight readings into less depth. Do the opposite for stations with clear deep water. This task is accomplished as follows:

i. Once on station, place the deck sensor in a position where it will not be shaded by any equipment or personnel.

ii. Add sufficient weight to the bottom ring on the lowering frame so that it will hang vertically in any currents.

iii. Working on the sunny side of the boat, suspend the lowering frame to the 0.1 m depth mark. Make sure that nothing is shading either of the sensors. Reposition the boat or yourself if water currents spin the lowering frame into a position that causes it to shade the underwater sensor.

iv. Once at the proper depth, wait for a minimum of 15 seconds while the running average is updated. Pay the cable in and out as necessary to cancel out any motion of the vessel and maintain a constant depth. If the sensor is accidentally raised or lowered during this time, you must wait another full 15 seconds from the time when the correct depth was reestablished.

v. Record the values displayed for channel I1 (PPFD units of µmoles photons $m^{-2}s^{-1}$) and channel M1 (% of incident PAR).

vi. Repeat this process at each of the remaining depths (e.g. 0.2 m, 0.4 m, 0.8 m, 1.4 m, and 2.0 m). If the bottom is reached before completing the above depth increments, lower the frame to the deepest possible whole increment marked on the rope and record the 15-sec averages from channels I1 and M1.

vii. All of the above data must be recorded on the Spatial Survey Station Data Sheet (Appendix 6) along with notations on the time (local), cloud cover (± 25%), wind condition and sea state (Beaufort Scale), and water depth. If adding or repeating depth readings at a station, log them in the sequence they were measured (not necessarily by increasing depth). If you choose to redo a reading, do NOT erase or write over the discarded value with the new one. Strike out and initial the suspect value, but do so in a way that maintains the legibility of the datum. It may still be used to help troubleshoot attenuation coefficients with poor correlation coefficients. Record the new datum in the next available free space.

A light profile must be measured at each of the spatial survey stations each time they are sampled. If the spatial sampling is conducted as designed, then 6 trend stations plus 6 random stations are occupied for each of four weekly survey cruises. Light profiles must also be measured at the continuous monitoring station to check the performance of the continuous PAR sensors and to provide data for a post-deployment calibration should it prove necessary. At a very minimum, a light profile must be measured at the time the sonde is deployed, retrieved, and during each of the four weekly spatial survey cruises. Review the SOP for continuous water quality monitoring to determine whether additional light profiles are needed. Light profiles are measured the same for the continuous monitoring station with the following additional requirements:

i. It is very important that the light profile be measured as closely in time as possible with a logging event on the continuous sonde. These logging events occur regularly on the quarter hour.

ii. It is very important that, in addition to the profiling PAR sensors, the PAR sensors on the continuous YSI sonde not be shaded by the boat or any other equipment or personnel.

2.6.5.1 *Equipment Maintenance, Handling and Precautions*

Although LiCor describes the LI-1400 as weatherproof, the O-ring that seals the case is deliberately cut by the manufacturer to allow water vapor out of the case. Consequently, water can get inside the case and damage the circuitry. You may wish to replace the "cut" O-ring with an intact one to resolve this issue. If you do, understand that you will need to keep packets of silica gel inside the case to remove any residual moisture. These should be recharged or replaced each time the case is opened to service the batteries. Also note that several of the pins on the outside D-subminiature connectors carry voltages. If these pins get wet, particularly with seawater, they can become short circuited to ground and corrosion will occur. Take appropriate precautions to ensure that rain and spray do not come in contact with the LI-1400. In wet field conditions you may choose to cap the 9 and 25-pin D-subminiature connectors and the channel I3 BNC connector with dummy plugs and or place the LI-1400 inside a clear vinyl waterproof camera bag. While the cables will prevent the bag from sealing properly, splash protection will still be afforded.

At the end of each field survey, rinse the underwater sensor and lowering frame with fresh water. If the deck sensor was splashed with seawater, it can also be gently rinsed with freshwater (do not use a high-pressure spray). The LI-1400 should be wiped clean with a wet sponge. Isopropyl alcohol can be used to clean and dry the BNC and D-subminiature connectors on the logger if they were severely splashed with seawater. The deck sensor is weatherproof and can withstand a light spray of fresh water, but should not be immersed. Be careful not to scratch the acrylic optical windows or damage the black paint within the annular rings on either of the sensors. LiCor ships its PAR sensors with protective caps, and these should be used whenever the sensors are in transit or storage. When cleaning the diffuser element on a PAR sensor, do not use alcohol, organic solvents, abrasives, or strong detergents. Mild dishwashing detergent and vinegar are acceptable cleaning agents.

2.6.6 *References*

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2.7 SOP 7 – Chlorophyll-*a* Sampling and Analysis

2.7.1 *Introduction*

This SOP describes the methods for collecting near-surface water samples, filtering them through glass-fiber filters, and analyzing them for chlorophyll-*a* and other pigments. *Chlorophyll-a* constitutes roughly 1-2% of the dry weight of phytoplankton, and consequently is a convenient proxy measure of phytoplankton biomass. Chlorophyll is measured *in situ* using a chlorophyll sensor (fluorometer) on the YSI sonde for spatial and continuous water quality monitoring (SOPs 4 and 5). Measurements from each sonde must, however, be calibrated against laboratory methods where chlorophyll is first extracted from the cells. A standard curve for the YSI chlorophyll sensor is generated by simultaneously measuring chlorophyll by *in situ* fluorescence (using the YSI sonde) and collecting a grab sample for laboratory extraction of chlorophyll. This is performed at each station, and must be done every time the sonde is recalibrated (i.e. for each of the four weekly sampling efforts). A standard curve is also required for the sonde deployed in autonomous mode at the continuous logging station. For this standard curve, discrete samples are gathered repeatedly at the logging station over the course of the deployment.

There are three basic approaches for the analysis of chlorophyll-*a* from phytoplankton. In each case, phytoplankton are collected on a filter and the chlorophyll is extracted in 90% acetone. The three methods are distinguished principally by their methods of detection: visible spectrophotometry, fluorometry, or high-performance liquid chromatography (HPLC, also known as high-pressure liquid chromatography). HPLC yields the most detailed information about the photosynthetic pigments in the sample, followed by spectrophotometry. Fluorometric methods have the advantage of being highly sensitive. This allows smaller volumes of water to be filtered and the option for an analytical laboratory to eliminate the step of filter grinding. For parks without existing monitoring programs, the spectrophotometric approach is recommended as the best compromise between cost and information for this protocol.

Standard methods are recommended for adoption at all parks. The EPA methods for chlorophyll-*a* analysis (Table 15), have been adopted as the NACP standards. These methods prescribe QA/QC requirements for the laboratory that include an initial demonstration of capability. For parks already measuring chlorophyll-*a* using method 10200 H from Standard Methods of Water and Wastewater Analysis, the laboratory need only add the quality control steps (section 9) from the corresponding EPA method (Table 15). Other analytical methods are not recommended and would require case-specific evaluation to ensure QA/AC and compatibility with NACP data.

Table 15. NACP-adopted methods of chlorophyll-*a* analysis.

Analytical Approach	EPA Method	Standard Methods of Water and Wastewater Analysis
Fluorometric	445.0	10200.H.3
Spectrophotometric	446.0	10200.H.2
HPLC	447.0	----------

As designed, the water quality monitoring protocol requires that 13 stations be visited each week during the four-week index period (six trend stations, six probability stations, and the continuous monitoring station). Several additional samples must be collected at the continuous station, the exact number of which depends on the number of sonde deployments during the index period (see the SOP for Continuous Water Quality Monitoring with YSI Sonde). Adequate supplies and material must be acquired to accommodate the collection, filtration and storage of 120 samples per park each season (samples are filtered in duplicate).

2.7.2 *Collection and Field Handling of Samples*

At each station, a 1 L water sample is collected from a depth of 20-30 cm. It is important that these discrete samples not be biased by pleuston and neuston floating at the water's very surface. This can be accomplished without the need for special sampling equipment by gathering water directly into the sample container. If there are concerns about potential contact with microbiological or chemical contaminants in the water, any style of sub-surface water grab sampler may be used.

2.7.2.1 *Equipment and supplies for sample collection*

- 14 1-L amber polyethylene wide mouth sample bottles
- insulated chest large enough to accommodate the above bottles arranged in an upright position
- 10-15 lbs of ice (cubes or crushed)
- optional sub-surface water sampling device

2.7.2.2 *Methods of sample collection*

Once on station, select a clean dry sample bottle and label it with the spatial element (i.e. hexagon) number and the time. If sampling at the continuous monitoring station, substitute "continuous station" for the number of the spatial element. Loosen the cap, but hold it in place on the mouth of the bottle. Invert the bottle and lower it to a depth of 20-30 cm. While holding at that depth, tip the bottle upright, allowing air to escape from under the cap. Loosely cap the bottle, bring it to the surface, agitate to rinse and dump the contents (away from the spot where you are collecting the sample). Repeat this two more times (three rinses total). Repeat this filling procedure a fourth time, but cap tightly to retaining the contents. Plunge the sample into an ice water bath in the insulated chest (0-4 ˚C). It is imperative that samples be kept cold and dark the entire time they are being held for filtering.

One 1-L grab sample must be collected at each of the spatial survey stations each time they are sampled. If the spatial sampling is conducted as designed, then 6 trend stations and 6 random stations are occupied for each of four weekly survey cruises. The 12 samples from each cruise will be used to calibrate the fluorometer for that cruise.

Grab samples must also be collected to calibrate the fluorometer on the YSI sonde at the continuous monitoring station. At a very minimum, a 1-L grab sample shall be collected at the time the sonde is deployed, retrieved, and during each of the four weekly spatial survey cruises. Review SOP 4 (Continuous Water Quality Monitoring with the YSI

Sonde) to determine whether additional grab samples are needed. For grab samples taken at the continuous logging station, it is very important that the time of collection coincide as closely as possible with a logging event of the continuous sonde. These occur regularly on the quarter hour.

2.7.3 *Sample Filtration*

Filtering should be performed in subdued light as soon as possible after sampling since algal populations, and thus chlorophyll-*a* concentration, can change in a relatively short period of time. The EPA recommended holding time of raw water samples prior to filtration is four hours provided the sample is held at 4 ˚C. This SOP calls for samples to be filtered under controlled laboratory conditions rather than in the field. While this may result in some samples being held for more than four hours, it has several advantages over filtering in the field that make it preferable for Vital Signs monitoring. These include better control over the volume of water being filtered, better control of pressure differential (vacuum) across the filter, ability to work under subdued light, and a shorter station time. This last factor has implications for other Vital Signs such as PAR attenuation that must be measured as close to local apparent noon as possible.

Minimize the amount of time that raw water samples are held by initiating the filtering procedures as soon as possible after returning from the field, and by filtering samples in the order they were collected.

2.7.3.1 *Equipment, lab ware and supplies for sample filtration*

- vacuum pump capable of maintaining a vacuum up to 20 KPa (6 in. Hg) and a meter or so of vacuum hose
- filtration apparatus consisting of a 1- or 2-L filtration flask, a 25-mm (or 47-mm) fritted glass vacuum filter holder with glass filter funnel, and a single-hole rubber stopper to connect the two.
- filters, 25-mm or 47-mm glass fiber, nominal pore size of 0.7 µm, such as Whatman GF/F filters.
- Petri dishes, plastic, 50 X 9-mm
- aluminum foil
- graduated cylinders, 250-mL and 500-mL
- tweezers or flat-tipped forceps
- dry ice or ultra-freezer (-20 ˚C to -70 ˚C)

2.7.3.2 *Filtering methods*

All reusable lab ware that comes in contact with chlorophyll solutions should be clean and acid free. An acceptable cleaning procedure is soaking for 4 h in laboratory grade detergent and water, and rinsing with tap water, distilled deionized water and acetone. Assemble the filtration apparatus and attach the vacuum pump via the vacuum hose. Filtering should be performed under very subdued light – the minimum that still allows the technician to perform the task safely.

Each water sample will be filtered onto two duplicate filters. A subset of these will be used for QA/QC purposed by the analytical lab where they are processed. The remainder provide a backup should the first sample analysis be spoiled. Proceed as follows for each water sample:

Place a filter, rough side up (woven side down), on the filter holder and install the filter funnel. It is important that the filter be properly centered on the holder and the funned be properly attached so that the sample cannot leak around the filter.

Prior to drawing a subsample of water, gently resuspend the particulate material by swirling it three times in each direction.

Pour off a subsample into a graduated cylinder and accurately measure the volume. Pour this subsample into the filter tower of the filtration apparatus and apply a vacuum. Vacuum filtration should not exceed 20 KPa (6 in. Hg). Higher filtration pressures or excessively long filtration times (>10 min) may damage cells and result in loss of chlorophyll. Experience and care will prevent you from attempting to filter excessively large subsamples, which clog the filter. Typically, a sufficient volume has been filtered for spectrophotometric and HPLC methods when a green or brown color is clearly apparent on the filter. For fluorometric analysis, filter just enough water to create a faint color on the filter. The fluorometric method is much more sensitive and if too much material is captured on the filter, the extraction efficiency will be reduced and chlorophyll will be underestimated.

Do not suck the filter dry with the vacuum. Instead, slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.

Remove the filter from the support/base with tweezers, fold once with the particulate matter inside, lightly blot the filter with a tissue to remove excess moisture and place it in a Petri dish. On the outside of the Petri dish, record the sample ID (same as on the water collection bottle), the date, the replicate number (rep1 or rep2), and the volume of water that was filtered. Also record these values on the Chlorophyll Filtering Data Sheet (Appendix 7). Wrap the Petri dish with aluminum foil to protect the sample from light, and re-label the outside of the foil identically to the Petri dish itself. Immediately store the sample at -20 ˚C to -70 ˚C in an ultra freezer or on dry ice.

Repeat this procedure for the second replicate then proceed on with the remaining water samples. Be certain to check the level on the filter flask regularly and empty as necessary (seawater may damage the vacuum pumps if it overflows the sidearm filter flask). Also be certain to check the vacuum level with each sample.

2.7.4 *Storing Samples and Shipping for Analysis*

Samples may be stored frozen at -20 ˚C for as long as 3.5 weeks without significant loss of chlorophyll-*a*. If chlorophyll is to be extracted and analyzed in-house, then follow guidance in the applicable EPA method (or SM 10200.H with added EPA QA/QC). In most cases, however, samples will be shipped to a contract laboratory. Prior to shipping, confirm that the receiving laboratory has appropriate sample storage facilities and alert them to expect a shipment requiring immediate attention upon arrival. Express ship all samples on dry ice using overnight service following applicable federal, state and local

shipping regulations for hazardous materials (dry ice). Federal Express will currently overnight ship samples packaged with dry ice at no additional charge over their standard rates. If a Styrofoam insulated box is used, it must be encased in an outer cardboard container. Small insulated coolers (with rigid shell) are also acceptable. All shipments containing dry ice must carry an appropriate hazardous material label (provided by FedEx).

2.8 SOP 8 – Sediment Total Organic Carbon (TOC) Sampling and Analysis

2.8.1 *Introduction*

This SOP covers the methods for collecting surface sediments from NACP estuaries and analyzing them for total organic carbon (TOC). Sediment TOC is measured every fifth year of the monitoring program. The same probability-based sampling design is applied to TOC sampling as is used for spatial water quality monitoring. This includes the requirement that all sampling be completed during a four week index period during mid-to-late summer (July-August). For logistical simplicity, it is recommended that TOC sampling be incorporated into the fieldwork for water quality monitoring. All aspects of this method follow exactly the methods prescribed for the US EPA National Coastal Assessment (NCA). It is highly recommended that NPS Vital Signs personnel familiarize themselves with the EPA NCA Field Operations Manual (US EPA 2001a) and Quality Assurance Project Plan (US EPA 2001b) as they relate to this SOP. Note that the above two EPA documents differ in their recommendations on sample storage. For Vital Signs monitoring, NPS is advised to follow the guidance from the latter EPA document and store and ship samples frozen at –20°C. Another important difference between this SOP and the NCA method is that the NCA protocol includes additional benthic measures and requires larger volumes of sediment. NPS sediment sampling for TOC requires only 100 ml of surface sediment (the top 2-3 cm) and may be accomplished with substantially lighter gear (as allowed by NCA).

2.8.2 *Collection and Field Handling of Samples*

2.8.2.1 *Station locations*

Every five years, NPS NACP monitoring shall include sediment sampling for TOC. Sediment samples are collected using the same probability based spatial design applied to water quality monitoring. At each park, 30 stations are occupied during a four-week late summer index period, and these stations shall be the same ones used for water quality monitoring for that sampling year. For logistical efficiency, it may be desirable to incorporate sediment sampling into water quality survey cruises, but this is not required. Nor is it necessary to repeat the sampling sediments at trend stations during each week of the index period. Only one composite sample is required for each station during the index period.

2.8.2.2 *Collection gear*

The standard sediment grab specified for NCA collection is a 1/25 (0.04) m^2, stainless steel, Young-modified Van Veen Grab; however other gear is also acceptable (US EPA 2001a). Since NPS Vital Signs Monitoring requires a relatively small amount of sample (100 ml) from a relatively shallow depth (2 cm), alternative coring devices are likely to be more efficient. Whichever collection gear is used, it must be capable of gathering a 7 cm deep sample with an undisturbed surface layer. In order to collect the required volume of sediment within 3 cores, the device should have a diameter no less than 5 cm. In addition to the sediment sampler, this SOP requires the following.

- 500 ml vessel with lid for homogenizing composite samples

- spatula or scoop
- wide-mouth glass sample jars (250 ml)
- ice and insulated chest
- cm ruler

While the NCA protocol calls for stainless steel or Teflon sampling devices, the constraint is not necessary for this SOP since organic contaminants are not being measured. Similarly, the requirement to wash all equipment with Alconox between stations may be omitted. Coring devices and utensils should, however, be rinsed clean of any residual sediment between stations.

2.8.2.3 *Sample collection*

At each site, three sediment grabs will be collected and the surficial sediment layer (top 2 cm) will be collected by spatula or scoop. The surficial sediments from these three grabs will be combined in a clean vessel. Between grabs, the vessel shall be held on wet ice (0-4 °C) and covered to prevent contamination. Once three grabs have been added to the vessel, the composite sample shall be well blended to ensure a homogenous mixture. Approximately 100 ml of the composite sample shall be placed in a clean, pre-labeled glass jar and held on wet ice until back at the laboratory, at which point the sample shall be frozen at -20°C until further analysis. It is recommended for this SOP that jars with a capacity of at least 250 ml be used since full jars tend to break when frozen.

It is imperative that only "successful" core or grab samples be included in the composite. A successful sample is one having a level, intact sediment surface over the entire area of the grab, and a total depth of at least 7 cm. Grabs containing no sediments, or grabs with shelly substrates or grossly slumped surfaces are unacceptable. Grabs with washed-out surfaces, or ones completely filled to the top (where it is not possible to confirm an intact sediment surface) are also unacceptable.

2.8.3 *Storing, Shipping and Analysis of Samples*

TOC in sediment samples is analyzed by the method described in US EPA 1995. Samples are dried and then acidified to remove any inorganic (carbonate) sources of carbon. A sub-sample is then combusted in a TOC analyzer using infrared detection of carbon dioxide gas. It is acceptable for this analysis to be conducted in-house, but in most cases a contract laboratory will be used. In either case, it is expected that the analysis will follow the NCA method (US EPA 1995) as well as the quality assurance procedures described in the NCA Quality Assurance Project Plan (US EPA 2001b). TOC is run in batches of 20-25 samples, and QC samples included with each bath are: method blank, at least one duplicated sample, and a sample certified marine reference material from the National research council of Canada's Marine Analytical Chemistry Standards Program (e.g.BCSS-1, MESS-2 or PACS-1). QC criteria and reporting requirements are provided in US EPA 2001b.

Until analysis, samples should be stored frozen at -20 ˚C. At this temperature, they can be held for up to one year. In most cases samples will be shipped to a contract laboratory. Prior to shipping, confirm that the receiving laboratory has appropriate sample storage facilities and alert them to expect a shipment requiring immediate attention upon arrival. Express ship all samples on dry ice using overnight service

following applicable federal, state and local shipping regulations for hazardous materials (dry ice). Federal Express will currently overnight ship samples packaged with dry ice at no additional charge over their standard rates. If a Styrofoam insulated box is used, it must be encased in an outer cardboard container. Small insulated coolers (with rigid shell) are also acceptable. All shipments containing dry ice must carry an appropriate hazardous material label (provided by FedEx).

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2.9 SOP 9 – Submerged Aquatic Vegetation (SAV) Mapping

2.9.1 *Introduction*

Guidance for mapping submerged aquatic vegetation (SAV) was developed for the NOAA Coastal Change Analysis Program (C-CAP). C-CAP is part of the Coastal Remote Sensing Program of the NOAA Coastal Services Center (CSC), located in Charleston, South Carolina. Benthic habitat mapping, which was originally part of the C-CAP function (Dobson et al. 1995), is now being developed separately within CSC and according to new data standards (see Finkbeiner et al. 2001, *Guidance for Benthic Habitat Mapping an aerial photographic approach*). Several existing mapping programs already provide information on the distribution of SAV in the majority of North Atlantic Coastal Parks (NACP). These programs all follow the NOAA standards.

The basic mapping process involved the following steps, the details of which are documented in the metadata for each of the mapping programs as well as in Finkbeiner et al. 2001.

1. *Acquisition of aerial photography*
2. *Photointerpretation of SAV resources*
3. *Fieldwork to confirm photo-interpreted features*
4. *Compilation to a digital base map*
5. *Independent accuracy assessment procedure*

Several of the existing mapping programs that overlap with the NACP are relatively new, and the frequency at which they will be remapped remains to be demonstrated. For those parks without SAV mapping, or where it is repeated in intervals of longer than five years, NPS will need to initiate additional mapping of its own. Where NPS mapping is to supplement less frequent state mapping, it is important that methods match with existing programs so data are comparable (particularly the photography scale and minimum mapping unit). Otherwise, it is acceptable to simply gather the GIS coverages from the individual mapping programs and examine the data for trends in SAV habitat loss or gain.

For cases where the NPS needs to initiate additional SAV mapping, the recommended approach is to partner with the NOAA CSC Benthic Habitat Mapping Project. This mapping project is an ongoing activity of CSC, which emphasizes the development of standards, collaborative partnerships, and technical guidance to the resource management community. In addition to their technical expertise, CSC maintains a list of qualified contractors and may soon have standardized "statements of work" available to NPS for use when contracting for mapping services (acquisition of aerial photography, photo interpretation, and field verification). Current points of contact at the NOAA Coastal Services Center for seagrass mapping are Bill Stevenson (bill.stevenson@noaa.gov, 843-740-1299) and Mark Finkbeiner (mark.finkbeiner@noaa.gov, 843-740-1264).

2.9.2 *Acadia National Park*

Maine's Department of Marine Resources (DMR) has mapped the SAV habitat for the entire state following C-CAP Protocols. This mapping was accomplished from aerial photography captured on conventional Kodak Aerochrome 2448 color positive film at a nominal scale of 1:12,000. The coast was flown in an incremental fashion from 1993 through 1997, and a composite coastal SAV dataset created from these multiple years of data. A polygon GIS coverage from this data (megrass) is available from the Maine Office of GIS at http://musashi.ogis.state.me.us/catalog/ along with the applicable metadata. In addition to mapping SAV bed outlines, habitat is categorized into five separate cover classes (see metadata). A goal of the ME Department of Marine Resources it to periodically update this coverage, but the area surrounding ACAD has not been remapped since the original aerial photography of 1996. Additionally, only the Somes Sound spatial domain was mapped by the state. The shallower systems of Northeast Creek and Bass Harbor Marsh were outside the area of the DMR's mapping program (although some photography from 1996 is available). For an inventory of DMR's coastal photography for Maine see http://www.state.me.us/dmr/aerialphotos/preview/preview.html.

NPS will need to either contract for its own SAV habitat maps, or investigate the feasibility of entering into a cooperative agreement with DMR to expand (in time and space) its mapping activities in this area. Since DMR's mapping effort addresses *Zostera marina*, it is important to note that *Ruppia maritima* is the SAV species found within the Northeast Creek spatial domain, and that both *R. maritima* and *Z. marina* are found in Bass Harbor Marsh. The current point of contact for SAV mapping at Maine DMR is Seth Barker (Seth.Barker@state.me.us, 207-633-9507).

2.9.3 *Boston Harbor Islands National Park Area and Cape Cod National Seashore*

The Massachusetts Department of Environmental Protection (DEP) Wetlands Conservancy Program (WCP) has an ongoing mapping program for SAV habitat in the state. The program was developed in collaboration with NOAA CSC using C-CAP protocols, and is accomplished from aerial photography captured on conventional Aerocolor 2448 color positive film at a nominal scale of 1:20,000. The coast was first flown in an incremental fashion from 1993 through 1996, and a composite coastal SAV dataset created from these multiple years of data. A polygon GIS coverage (eelgrass) is available from the Massachusetts Office of GIS at http://www.mass.gov/mgis/ftpstate.htm along with a point coverage for the field verification points (http://www.mass.gov/mgis/eelgrass.htmegrasvpt) the applicable metadata for each.

In addition to mapping SAV bed outlines, habitat is categorized as either *R. maritima* or *Z. marina*. The goal of the Mass. DEP WCP is to update all maps on a recurring 5-year interval. Photography covering BOHA and CACO was originally captured in 1995, then again for CACO in 2000 and for BOHA in 2001. The more recent coverages are also available from Mass GIS, but not as part of the 1999 composite. This program should continue to meet the SAV mapping information needs for this protocol; however

continued communication will be required. The current point of contact for SAV mapping by the Commonwealth of Massachusetts is Charles T. Costello, Section Chief, DEP WCP (charles.costello@state.ma.us, 617- 292-5907).

2.9.4 *Fire Island National Seashore, Gateway National Recreation Area, Sagamore Hill National Historic Site*

No ongoing benthic mapping programs are in existence that would be relevant to SAV resources at SAHI or GATE. Although SAHI enjoys only limited water frontage on Cold Spring Harbor, much of the harbor substratum (and the greater Oyster Bay system) falls within the boundary of the Oyster Bay National Wildlife Refuge, which is largely an estuarine subtidal refuge. Also in the vicinity is the Target Rock National Wildlife Refuge. The presence of additional DOI land interests in the area presents an opportunity to collaborate and cost share for benthic mapping.

Although eelgrass is not known to exist at the Jamaica Bay or Staten Island Units of GATE, there are literature reports of eelgrass in the Navesink River near the Sandy Hook Unit (Kuropat et al. 2002, Phelan et al. 2000), but these have not been systematically mapped, and there are no recent report of eelgrass within or adjacent to park boundaries. Nevertheless, baseline maps using a consistent aerial photographic method are needed in order to document any future expansion of SAV resources in the park. Until such a time when eelgrass is seen to be recolonizing the system; however, it is reasonable to remap on much reduced (decadal) intervals.

In Great South Bay on Long Island, the NOAA CSC recently assisted the New York Department of State's Division of Coastal Resources office (NYDOS) and local government agencies to create a baseline SAV map for Long Island's South Shore Estuary Reserve (SSER). This baseline inventory is a key element of the Comprehensive Management Plan developed for the reserve and is intended to be used for change detection in the future. The frequency at which this system will be remapped remains to be demonstrated, so it may be necessary for NPS to supplement with additional mapping, or partner with NYDOS and the SSER in order to ensure a frequency of no more than every five years. NPS may also need to expand SSER mapping to include portions of Narrow Bay and Moriches Bay that fall outside the reserve.

The current SSER mapping effort used conventional-color metric aerial photography. Photographs with a map ration of 1:20000 were acquired in June of 2002 in order to avoid problems with poor water clarity associated with the late summer in this system. Habitat mapping and ortho mosaic development were accomplished by soft-copy photogrammetric methods. CSC staff conducted initial fieldwork to support the image interpretation and conducted a field validation of the final SAV map in the early fall of 2003. A vector GIS coverage from this data (final-poly) is available from the NY State Department of State at http://www.nysgis.state.ny.us/inventories/state.htm along with the applicable metadata. Current NYDOS point of contact for SAV mapping in this region is Fred Mushacke (fmmushac@gw.dec.state.ny.us, 631-444-0465).

2.9.5 *Assateague Island National Seashore, Colonial National Historical Park, George Washington Birthplace National Monument*

The Maryland and Virginia NCBN parks benefit from a longstanding SAV mapping program which, in recent years, has been updated annually. The program is run by Robert Orth at the Virginia Institute of Marine Science (804-684-7392, jjorth@vims.edu), and detailed information on mapping methods, years of coverage, and data are all available online at http://www.vims.edu/bio/sav/. Chesapeake Bay and Coastal Bays SAV have been mapped from aerial photography, primarily at a scale of 1:24,000, for the following regions: western shore, Va. only - 1971 & 1974; lower Bay, Va. only - 1980 & 1981; upper Bay, selected sections, 1979; Baywide, 1978, 1984 - 1987, and 1989 - 2002. Each area of SAV is classified into one of four density classes by the percentage of cover as determined from the aerial photography methodology described in each annual report - *e.g.* Orth et al. 2003. Current methods involve the use of aerial photography on black-and-white Agfa Pan 200 film at a scale of 1:24,000. Images are scanned, georectified and orthographically corrected to create orthophoto mosaics. Outlines of SAV beds are interpreted on-screen and edgematched. Ground surveys are then used to field check the photo-interpretation. The final product is a digital database for analysis of bed areas and locations.

To comply with the need for consistency, quality assurance, and quality control, guidelines are in place for the acquisition of aerial photography, and standard operating procedures used for orthorectification, mosaicing, photo-interpretation, edgematching, and field verification. This program should fulfill the information need for NPS SAV mapping for the foreseeable future. GIS polygon coverages for each year of the program are available at http://www.vims.edu/bio/sav/savdata.html along with the applicable metadata.

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2.10 SOP 10 – Seagrass Condition

2.10.1 *Introduction*

This SOP describes measurement of seagrass structural characteristics used as early indicators of ecosystem condition in response to nutrient enrichment. The monitoring protocol is based on population- and shoot-based measures of seagrass condition, as well as important ancillary variables that influence seagrass responses to nutrient load. Procedures are derived from the SeagrassNet protocol for global seagrass monitoring (www.seagrassnet.org), a program designed to investigate and document the status and trends of seagrass resources around the world. The program was originated in 2001 in the Western Pacific, where 19 monitoring sites were established in seven countries. Since then, three SeagrassNet sites have been established in Belize, four in Brazil, and four in the United States. Two of the US sites were established at Cape Cod National Seashore during development of this SOP. We have adapted SeagrassNet methods (Short et al. 2004) for use in North Atlantic Coastal Parks (NACP) to accommodate regional characteristics of seagrass ecosystems and logistical constraints in implementation. The NPS procedures for monitoring seagrass condition are consistent with the global SeagrassNet program in fundamental aspects of sampling design, measures, and sampling methods, so that NPS data may contribute to the global SeagrassNet database. Use of methods compatible with SeagrassNet provides a global framework for interpretation of NPS monitoring data.

SeagrassNet monitoring is based on repeated measurement of permanent quadrats located along three 50-m transects that are positioned parallel to shore and along a depth gradient. The sampling objective is not to describe trends in condition of an entire seagrass bed, which would require a large sampling investment, but rather to describe changes in seagrass characteristics over time at locations that are representative of the seagrass depth range. In general, seagrass responses to nutrient enrichment will be detected first at the deepest locations of existing beds (cf. Background and Objectives 1.1.4.6). Consequently, comparisons of trends in seagrass characteristics between shallow and deep locations can be helpful in forecasting seagrass declines. This approach to monitoring seagrass condition provides high resolution information that complements large-scale measurement of seagrass distribution (SOP 9, SAV Mapping). Random placement of sampling quadrats along transects ensures unbiased measurements at each transect location, and repeated measurement of specific quadrats provides the maximum ability to detect changes over time.

Sampling is conducted at extreme low tides to minimize water depths. Snorkeling ability is required to sample some of the quadrats; although use of SCUBA would facilitate sampling the deepest quadrats, the methods presented here have been designed for implementation with snorkeling alone. The following methods are drawn extensively from the SeagrassNet monitoring manual (Short et al. 2004), which are based on an extensive compilation of seagrass research techniques for global application (Short and Coles 2001). NPS personnel involved in monitoring seagrass condition should refer to the SeagrassNet manual for an overview of the global program and more detailed descriptions of methods where noted.

2.10.2 *Equipment and Supplies*

The following equipment and supplies are used for within-bed seagrass monitoring:

- plant press and herbarium supplies (acid-free herbarium mounting cards, museum-quality mounting glue, herbarium specimen labels)
- 50-m measuring tape (3)
- 100-m measuring tape (2)
- compass
- 0.25 m^2 (50 cm x 50 cm) stainless-steel quadrat with washers welded in the middle of each edge as guides for subdivision rods
- 75-cm long orange fiberglass rods with tapered ends for subdividing quadrats into 4 0.0625-m^2 sections (2)
- 0.25 m^2 photo quadrats made of ½-inch CPVC
- long orange fiberglass rods for temporary station markers
- flagging tape
- cable ties, 8-inch black UV-stabilized
- side cutters for clipping cable ties
- meter stick
- digital land camera
- digital underwater camera
- floating plexiglass view frame, 60 cm x 60 cm (plexiglass attached to 5-cm deep wood frame that is covered with foam rubber)
- percent cover standard
- GPS unit, WAAS enabled
- permanent station marker (32-inch helical screw anchor, 15)
- crow bar, thick dowel, or other tool for installing screw anchors
- buoys (6)
- recording thermometer, Stowaway® TidbiT® or iButton® temperature logger (2)
- optic base station for launching TidbiT® temperature logger **OR**
- Blue Dot Receptor, 1-wire to RS-232 Adapter, optional USB to 1-wire adapter, and iButton Viewer software for equipment for launching iButton® temperature logger (see SeagrassNet manual for instructions)
- salinity meter or refractometer
- small bottles for salinity samples (3)
- light logger, Hobo® LI light logger with clear submersible case, desiccant packs (3 sets)
- wooden closet rod with two holes drilled in one end for mounting light logger on land
- Onset BoxCar® Windows software for logger management, with RS-232 connector cable and optional USB Serial adapter
- garden clippers
- plastic or stainless steel corer, 6.7-6.8 cm internal diameter, with end cap or stopper
- 20cc syringe sediment sampler, with rubber stoppers to seal
- dive slate with attached pencil
- mask, snorkel, booties, wet suit, weight belt (plus dive gear if using

SCUBA)
- nylon sample-bags, 1/16-inch mesh (36)
- insulated chest
- plastic bags (gallon-3; quart-12)
- plant sorting trays
- forceps for sorting plant samples
- glass microscope slides
- drying oven
- paper bags for drying plant samples (small Plain Kraft "merchandise bags")
- data sheets copied on waterproof paper for Cross transect A, Cross transect B, Cross transect C, and Light and Temperature Sensor Deployment Log (Appendix 8)
- pencils, permanent markers
- clip board
- video camera and equipment

2.10.3 *Establishing the Monitoring Station*

The site selected for monitoring seagrass condition at a given park should be representative of the park's seagrass resources. The site should reflect the depth range where seagrasses are typically found, and should not be exceptional in any characteristic. Ideally, seagrass distribution at the site should be fairly even, without large bare patches or physical disruptions such as tidal channels or sand bars. Homogeneity of the seagrass bed reduces long-term variability in sampled parameters and enhances the likelihood of detecting responses to nutrient enrichment. One of the most important considerations in site selection is accessibility; monitoring personnel must be able to access the site easily and safely by land or by boat. To ensure long-term monitoring of effects of estuarine nutrient enrichment, the site should be free of obvious large human impacts (e.g. dredging, boat moorings) that could disturb or eliminate seagrass habitat at that location.

Establish a transect perpendicular to the shore at the site determined to be the most appropriate for monitoring. The transect should begin about 1 m inside the continuous seagrass meadow. Mark the beginning of the transect with a temporary marker (fiberglass rod flagged with fluorescent tape); this will be Station A. The global SeagrassNet protocol calls for the seaward end of the transect to be located approximately 1 m inshore of the deep edge of the continuous seagrass bed. However, for NPS implementation, sampling logistics must also be considered in locating the transect's seaward end, as the deep edge of the seagrass beds in this region is often too deep to be sampled by snorkeling alone. If personnel are qualified and authorized to use SCUBA, then the seaward end of the transect can be located near the deep edge of the seagrass bed. If sampling will be done by snorkeling, then the deep end of the transect should be located at the deepest point that is comfortably and safely accessible by snorkeling (typically waist- to chest-deep at mean low water). Mark the end of the transect with a temporary marker (fiberglass rod or weight with rope and float attached, depending on the depth); this will be Station C. Identify a location on the transect that is mid-depth between Stations A and C and mark it with a temporary marker; this will be

Station B. Stations A, B, and C should now fall on a straight line that is perpendicular to the shore.

Three 50-m cross transects will be established along the shore-normal transect delineated by the temporary markers. Establish cross-transect A at the shallow location that was identified about 1 m inside the continuous meadow. Screw a permanent marker (screw anchor) into the sediment so that only "eye" at the top of the shank remains above the sediment. This is the center point for cross-transect A (i.e. position A-25, at a distance of 25 m along the 50-m cross transect). Use a tape measure to lay out the cross-transect so that it is 50 m long, roughly parallel to the shore, with the marker A25 at the center and following the same depth contour as closely as possible along the entire length. This will require three people, one stationed at the center and one on each end, to be sure the cross transect is straight, exactly 50 m long, with A25 at the exact center. Install permanent screw anchors at each end of the cross transect (A0, located at the left end of the cross transect when facing the sea, and A50, located at the right end of the cross transect when facing the sea). Record the GPS positions for the three permanent cross-transect markers and record the compass bearing of the cross transect from A0 to A50. Note that future sampling will occur by securing a tape measure to the permanent screw anchors, and locating permanent quadrat locations by their distances along the tape measure. In high energy environments it is advisable to install additional screw anchors for tape measure attachment midway between A0 and A25, and midway between A25 and A50, to reduce potential bowing of the tape measure along the 50-m cross transect. Short (ca. 50 cm) lengths of polypropylene line can be attached to the exposed end of the screw anchors as visual aids for relocation. Finally, install an extra screw anchor at each end of the cross transect, in line with the cross transect and 1 m away (to the outside of the cross transect). These screw anchors can be marked with buoys to assist in relocating the cross transect; extra screw anchors are installed for buoy attachment so that attention is not drawn to the actual cross transect markers. Record the GPS positions of the extra screw anchors for relocation purposes as well. Repeat this procedure for cross-transects B and C, at previously identified Stations B and C. Note that when all three cross transects have been established, their compass bearings should be the same.

2.10.4 *Sampling Frequency*

Seagrass monitoring should occur quarterly (April, July, October, January) during at least the first year of monitoring to establish baseline seasonal trends in condition measures. If possible, two years of quarterly monitoring provides information on annual variability in these trends. The minimum monitoring frequency thereafter is once per year, although continued quarterly sampling is advantageous and advisable if resources allow. Global SeagrassNet monitoring is conducted quarterly in perpetuity. Annual monitoring of seagrass condition in the NACP should occur in July, which is at or near the time of maximum seagrass biomass in this region (Roman and Able 1988, Kinney and Roman 1998, Short et al. 1993). July sampling also coincides with one sampling period of the global program, ensuring that NPS data are compatible with SeagrassNet and can contribute to the global database for interpretation within a broader context.

2.10.5 *Re-establishing the Cross Transects for Sampling*

Monitoring should occur in the shallowest tides possible during daylight hours. Use reliable tide tables (e.g. NOAA tables at http://co-ops.nos.noaa.gov/tide_pred.html) to identify predicted daytime low spring tides for sampling. Depending on the site logistics and number of personnel, it may require two low tides to sample all three cross transects. Monitoring personnel should arrive on site 1.5-2.5 hours before the predicted time of low tide to allow sufficient set-up time; sampling should begin as soon as water depths are workable, usually within 1-2 hours before low tide.

After locating the monitoring site, find the ends of the cross transects using buoys and/or GPS. Mark the ends of shallow transects with fiberglass rods and flagging tape to make transect work easier. Clean any algal growth or other fouling material off the permanent markers. Lay out a 50-m tape measure at all cross transects to be measured that day. Attach the measuring tape at "zero" to the transect screw anchor at the left side of the cross transect when looking at it facing the sea. Stretch the tape through the middle screw anchor (the Station marker) and attach it to the transect screw anchor at the right side of the cross transect (facing the sea). Remember that the screw anchors that mark the ends of the cross transects are 1 m toward the transect from the buoyed site markers. It is essential that the tape measure be stretched taut between the ends of the cross transect, as distances are used to relocate permanent quadrats.

Over five-year to decadal time scales, the edge of the seagrass meadow at permanent station A or C may shift. If either the shallow or deep edge of the bed has shifted so that the entire cross transect no longer has seagrass, establish a new Station A' or C' approximately one meter inside the new edge of the continuous bed. Conduct the sampling from cross transects based on these new locations and note the change on the data sheet. Measure and record the distance and depth differences from A to A' or C to C'. Record on the data sheet that there is no seagrass present on the original cross transect.

2.10.6 *Station Measures*

The following observations and measurements are taken during each monitoring event to characterize the environmental conditions at the depth stations.

2.10.6.1 *Monitoring Event Details*

General information about the monitoring event is recorded at the top of each cross-transect data sheet. Record the park unit, site name, sampling date, local time that sampling was initiated, personnel, weather (sky and wind conditions), predicted time and height of low tide with location of NOAA tide station used for reference, and any noteworthy comments (special conditions or observations).

2.10.6.2 *Light Level*

Onset-Hobo LI light loggers (www.onsetcomp.com) are used to measure and log light levels at the shallow (A) and deep (C) monitoring stations as well as at an unobstructed location above the high tide mark in the vicinity of the monitoring site (surface light, termed "Air" station). Hobo LI light loggers measure light intensity as lumens/ft^2 over a much broader spectrum than the photosynthetically available wavelengths (PAR, 400-700

nm). The Hobo LI loggers are designed to yield comparative data on light intensity in different settings rather than the absolute amount of incident light at any location. The logger is deployed inside a plastic case that filters out much of the light at high wavelengths, and the variables of interest are derived from the relative light measurements between two loggers (percent of surface light reaching A and C stations, and attenuation of light between the depths of A and C). Although the absolute Hobo LI intensity readings are not directly comparable to the amount of light measured by Licor PAR sensors (continuous and spatial monitoring SOPs 4 and 6), these derived variables are comparable to data obtained using PAR sensors. In addition, the low cost and small size of the Hobo LI loggers offer distinct advantages for monitoring the light environment at seagrass monitoring sites. In particular, no platform or infrastructure is needed to support or secure the logger; the entire unit is placed underwater, and so does not attract attention to the monitoring stations.

Hobo LI light loggers are deployed for one week immediately before or after each monitoring event. An extra trip to the monitoring site is required either a week before the monitoring event (to install loggers that will be retrieved during seagrass monitoring) or a week after (to retrieve loggers that were installed during seagrass monitoring). The deployment interval is dictated by the length of time the loggers remain sufficiently free of fouling organisms that accurate light measurements are not impeded, based on pilot tests at Cape Cod National Seashore. If initial assessments show that the loggers remain unfouled for an entire week, then the deployment interval could be extended to two weeks.

2.10.6.2.1 Launching the Light Loggers

Light loggers must be launched before deployment using Onset's BoxCar® (basic Windows® package for logger management) or BoxCar® Pro (includes advanced graphing and analysis functions) software. Launching should be done as close to deployment as possible to minimize collection and storage of meaningless data (i.e. values logged before deployment). Additionally, it is essential that all three loggers are launched as close to the same time as possible. Ultimately, the variables derived from the logged light data require relating light at each depth station to light at the surface (percent surface irradiance), and light logged at station A to light logged at station C (attenuation coefficient). These variables would be most accurate if all three loggers recorded light levels simultaneously. This is unrealistic given the time required to launch a single data logger (ca. 30-45 seconds) and the data logger characteristics; data loggers synchronize with the computer's internal clock upon launching and logging begins immediately (although the software includes a "multiple launch" option, this is not supported by the LI light loggers). However, with practice, all three loggers can and should be launched within a two minute interval, so that light data are logged at each station at nearly the same time. Record the date and time of launching on the Light and Temperature Sensor Deployment Log.

Follow the steps below to launch the light loggers.

- Open the BoxCar® software package on your computer.
- Connect the first Hobo LI light logger to the computer using the interface cable provided with the software. Note that the cable is equipped with a

9-pin RS-232 female connector for direct connection to the computer's serial port. If your computer has only USB ports then a USB Serial adapter (available from Onset®) will be necessary to connect the Hobo LI logger to the computer.

- Select "Launch" from the Logger menu. A dialog box will appear.
- Enter the appropriate logger label in the Description field. The label should include the 4-letter park unit code, site name, and station label (A, C, or "Air").
- Select a 10-minute logging interval.
- Select "Intensity (L/sf)" as the measurement unit.
- Be sure that "Wrap around when full" is not checked (appears as an "Advanced Option" in BoxCar®, and at the bottom of the dialogue window in BoxCar® Pro). This option instructs the logger to continue logging when the memory is full by overwriting the oldest data. If sensor retrieval were delayed, this could result in loss of meaningful data. If this option is not selected the logger will stop logging data when the memory is full.
- Select "Start". A dialog box will appear with the status of the launching process.
- Disconnect the logger from the interface cable before selecting "OK".
- Ensure that the red light in the corner of the logger is blinking, as this indicates that the logger is recording data.
- Repeat this process for the remaining two loggers in rapid succession, so that the recording intervals occur at approximately the same time for all loggers.

2.10.6.2.2 Deploying the Light Loggers

The sensors must be secured in clear submersible cases before deployment. Label each sensor with the appropriate identification (4-letter park unit code, site name, and station name) as well as the name and contact information of monitoring staff (e.g., Property of NPS, If found contact *staff person*, *telephone number*). Before inserting the logger in the case, it is necessary to angle the long axes of the bottom of the logger by light sanding; this changes the logger from a rectangle to a broad "U" shape in cross section and permits the logger to fit securely in the case. Remove the O-ring from the case and apply a thin film of silicon grease onto its surface with your fingertips. You should apply a very small amount of grease, just enough so the O-ring appears shiny. Replace the O-ring and slide the logger into the long, smooth end of the case with the light sensor on the side opposite the case's D-ring. Be sure that the logger sits horizontally in the case, perpendicular to the D-ring. Insert two small packets of dry silica gel in the case beneath the logger. The silica gel is blue when dry and pink if damp. The gel can be redried by placing the packet in an oven at 110-180^{o} C. Screw the two sections of the case firmly together, being careful not to over tighten.

In the field, locate the permanent screw anchor placed 1 m from the zero end of cross transect A (i.e., to the left of the cross transect when looking seaward). Typically this screw anchor will be marked with a buoy, which will facilitate logger retrieval. Clip the leaves of any seagrass within about a 1-m radius of the marker to prevent shading of the

light logger by the seagrass canopy. Verify that the label and serial number of the logger are intended for deployment at Station A and initial the field check box on the data sheet. Attach the case (containing the LI light logger and silica gel packs) to the screw anchor using four cable ties. The case should be positioned with the logger-containing end pointing south; ensure that the light sensor itself is horizontal and facing directly skywards. Buoyancy of the case requires that cable ties be positioned carefully to keep the housing horizontal. Record the date and time of deployment on the Light and Temperature Sensor Deployment Log. Repeat this procedure for the C and Air stations using the appropriately labeled loggers. The Air logger should be mounted on a pole that is long enough to elevate the logger above any ground vegetation. Note that the air logger is installed in the same type of clear case as are the submerged loggers.

2.10.6.2.3 Collecting the Light Loggers

Locate the permanent station markers with loggers attached and cut the cable ties to release the logger cases. Place the loggers in small plastic bags labeled with the logging location and record the date and time of collection on the data sheet. Rinse the case in fresh water and dry thoroughly before opening. Remove the case and remove the light logger. See section 2.10.10 for instructions on downloading data from the logger. The case can be cleaned with a dilute acid (10% hydrochloric acid or vinegar) solution to remove fouling. Do not scrub the case with anything abrasive as it will scratch the plastic. The cases can be reused as long as they remain clear. They will yellow with age, at which time they should be replaced.

2.10.6.3 *Temperature*

Water temperature is measured continuously at the shallow (A) and deep (C) monitoring stations. Temperature loggers are deployed and retrieved during each logging event. The duration of an individual logging interval is determined by how frequently monitoring occurs. Temperature and measured and logged with the Onset Stowaway TidbiT® temperature logger or the iButton® temperature logger. The iButton® logger is less expensive, but due to production limitations may be unavailable when needed. Although the Stowaway TidbiT® loggers are more expensive, we have never found them to be backordered. Instructions below relate to the Stowaway TidbiT® logger. Detailed instructions for launching and downloading the iButton® logger is provided in the SeagrassNet manual (Short et al. 2004).

2.10.6.3.1 Launching the Temperature Loggers

Stowaway TidBiT temperature loggers must be launched before deployment using Onset's BoxCar® (basic Windows® package for logger management) or BoxCar® Pro (includes advanced graphing and analysis functions) software. Connect the Optic Base Station to your PC using the PC interface cable provided with the software. Note that the cable is equipped with a 9-pin RS-232 female connector for direct connection to the computer's serial port. If your computer has only USB ports then a USB Serial adapter (available from Onset®) will be necessary to connect the Optic Base Station to the computer. Record the date and time of launching on the Light and Temperature Sensor Deployment Log.

Follow the steps below to launch the temperature loggers.

- Open the BoxCar® software package on your computer.
- Connect the first TidbiT® onto the TidbiT® coupler attached to the Optic Base Station.
- Select "Launch" from the Logger menu. A dialog box will appear.
- Enter the appropriate logger label in the Description field. The label should include the 4-letter park unit code, site name, and station label (A or C).
- Select a 30-minute logging interval.
- Select "Temperature (^{o} C)" as the measurement unit.
- Be sure that "Wrap around when full" is not checked (appears as an "Advanced Option" in BoxCar®, and at the bottom of the dialogue window in BoxCar® Pro). This option instructs the logger to continue logging when the memory is full by overwriting the oldest data. If logger retrieval were delayed, this could result in loss of meaningful data. If this option is not selected the logger will stop logging data when the memory is full.
- Select "Delayed Start"
- Enter the date and time to begin logging. Entering a time shortly before you plan to deploy the logger avoids accumulation of meaningless data that would have to be culled from the record.
- Ensure that Multiple Sampling is "Off". (Multiple Sampling causes the logger to make multiple measurements during the logging interval and store only the minimum, maximum, or average of the readings).
- Select "Start". A dialog box will appear to ensure that you want to launch the logger, as this will erase all previously collected data from the logger. Confirm that all data on the logger have already been downloaded and saved to a computer file before selecting "Yes".
- Disconnect the logger from the interface cable before selecting "OK".
- The green light should blink every four seconds, indicating that the logger has been launched and is waiting out the time delay. When logging has begun the green light will blink every two seconds.
- Repeat this process for the second temperature logger.

2.10.6.3.2 Installing and Retrieving the Temperature Loggers

The temperature loggers are attached to the permanent screw anchor placed 1 m from the zero end of cross transects A and C (i.e., marker to the left of the cross transect when looking seaward). These are the same screw anchors to which the light loggers are attached. Deploy the temperature logger by securing it to the screw anchor with a cable tie just above the sediment-water interface. This position ensures that the sensor is not exposed to air unless the seagrass bed is completely drained. Every time the station is monitored, each temperature logger is removed and replaced with a freshly launched logger. Place the retrieved logger in small plastic bags labeled with the logging location and record the date and time of deployment and retrieval on the Light and Temperature Sensor Deployment Log.

2.10.6.4 *Salinity*

Salinity is measured once during the monitoring event at the center permanent station marker (A-25, B-25, and C-25). Salinity should be measured close to the same tidal stage at all three stations when the entire site is submerged. Salinity can be measured *in situ* by transporting a salinity meter equipped with a submersible probe to the station (e.g. YSI 30 System instrument) or by collecting a small water sample for subsequent measurement. Regardless of approach, it is important that salinity is measured from a well-mixed area within the seagrass canopy rather than from the water above the canopy. To use a salinity meter *in situ*, lower the salinity probe into the seagrass canopy before recording the salinity. Alternatively, collect a water sample from the right depth by submerging a collection bottle with the lid on into the seagrass canopy, removing the lid to fill the bottle, and replacing the cap before raising the bottle. In this latter case, the salinity should be read as soon as possible after the sample is collected, using either a salinity meter or a refractometer. Instructions for proper use of a refractometer are provided in the SeagrassNet manual (Short et al. 2004).

2.10.7 *Cross Transect Measures*

The following measurements are made to characterize the structure of the seagrass bed and the environmental conditions along the cross transects.

2.10.7.1 *Distance to Shallow Seagrass Edge and Last Shoot*

Using a 50- or 100-m tape measure as needed, measure the distance onshore from the left (A-0), center (A-25), and right (A-50) cross transect positions perpendicularly to the limit of the seagrass bed. Two measures are taken to define the extent of the seagrass growth at the time of sampling. Distance onshore to edge of bed (m) is measured as the distance to the farthest point where shoots are less than or equal to one meter apart. Distance onshore to the last shoot (m) is measured as the distance to the most inshore shoot from cross transect A. Record these distances on the appropriate cross transect data sheet in meters to the nearest tenth of a meter. If the edge of the seagrass has contracted inside the position of the cross transect, record the distance as negative. In beds with dynamic edges, it may be helpful to sketch a diagram of the site on the back of the data sheet, specifying where the inner and outer edges of the meadow are located.

2.10.7.2 *Distance to Deep Seagrass Edge and Last Shoot*

Conceptually, these measurements are analogous to those described in the previous section. The goal is to record the deep edge of the bed by measuring its distances from the deep permanent cross transect. Unlike the shallow edge, however, these distances may be quite long and measuring them with a meter tape will not be feasible. Instead, distances are measured using WAAS enabled GPS and underwater videography. Distance are measured offshore from the Left (C0), Center (C25) and Right (C50) reference marks of cross transect C. Note that only the deep edge of the bed is measured, not the last shoot.

It is assumed that a small boat will be used for this task, so consistent methods for navigating the craft along the desired offshore course become important. To identify the proper offshore course, use a computer graphing application to plot the latitudes (Y-axis)

and longitudes (X-axis) of reference marks A0, B0, and C0. Use the coordinates already collected for these points, converting them into units of decimal degrees. Fit a least squares linear regression to these points and record the slope of this line. Repeat this process for reference marks A25, B25 and C25; and then again for A50, B50, and C50. Calculate the average slope of these three offshore trajectories. Note that these calculations assume a flat earth, a reasonable assumption for all distances anticipated during application of this SOP.

Using the average slope of these offshore trajectories, project a line from reference mark C0 and calculate points that fall along this line that are no farther than 25 m apart from each other. The coordinates for these points, listed in order, become "waypoints" along a "route" that can be piloted using navigation features commonly found on even the most inexpensive GPS units. Repeat the process to create offshore navigation routes for C25 and C50 using the same average slope from the previous paragraph.

Using a GPS with these three programmed routes, it is a relatively simple matter to navigate a small boat offshore from C0, C25 and C50. The seagrass bed is observed using an underwater drop camera, through which the deep edge of the bed is identified. Once identified, latitude and longitude of this edge are recorded, so that offshore distance can be calculated between it and the reference mark. This last step sounds much easier than it is; patchiness and holes in the bed make it difficult, and the bed edge is always passed before it is recognized. To make this task easier, and to establish a permanent visual record of the bed, we recommend using an underwater video drop-camera with the ability to record the video stream and integrated overlay of GPS data (latitude, longitude, date, time, speed, heading). This way data can be collected rapidly in the field, then the bed edge position evaluated more carefully back in the laboratory. For pilot testing we assembled one such camera system (Figure 18) for a total cost of under $1600 (Table 16). The selected camera also included on-screen data for temperature and depth.

Figure 18. Screen capture from underwater video taken at Cape Cod National Seashore using the described video equipment.

Table 16. Components and prices for a waterproof drop camera system with integrated waterproof deck unit consisting of a display, a digital recording device, a WAAS enabled GPS unit, and a video overlay unit to project GPS data onto the recorded video.

Aqua-Vu 120' DT video camera	$632
Black Box Camera GPS Video Overlay Controller	$126
Sony DCR-TRV460 Digital-8 HandyCam	$420
Garmin eTrex Legend GPS	$200
Waterproof Pellican Case #1450	$95
Garmin GPS Marine Mount	$36
CableClam waterproof bulkhead cable pass-through	$20
Plexiglass window and other miscellaneous parts	$70

2.10.7.3 *Depth*

Water depth is measured at the 0-, 25-, and 50-m positions of cross transects A, B, and C. Depth must be measured at each of the nine locations as close to the same time as possible, when the entire site is submerged. If any of the cross transects are intertidal, wait until all are submerged before measuring depth. Recording the depths of all locations at approximately the same time allows determination of the relative depth differences among sampling locations without introduction of error from tidal variation between measurements. Using a depth gauge (e.g., meter stick, long pole marked with depths, weight on a rope marked with depths, etc.) measure the depth from the top of the substrate at each location. Record the depth and time on the datasheets.

2.10.7.4 *Surface Sediment Observation and Sample*

Assess the sediment at the 0-, 25-, and 50-m positions of each cross transect by feeling the texture of the top two centimeters of substrate. Classify the sediment as one of the following: mud, fine sand, sand, coarse sand, shell, gravel, or rock. Record the sediment type in the three appropriate fields on each cross transect data sheet.

Sediment samples are collected from the 0-, 25-, and 50-m positions of each cross transect with a 20-cc syringe corer (plastic syringe with graduated tip cut off flush with the zero volume mark). Place the cut off end of the syringe barrel at the sediment surface, hold the plunger at a constant height at the sediment surface, and push the barrel into the substrate. Gently extract the sediment-filled syringe from the substrate and cap the cut off end with a rubber stopper. Empty the entire contents of the syringe into a small plastic bag labeled with the sample location and date. Close the bags tightly,

keeping as little air in the bags as possible. Indicate with check marks on the data sheets that sediment samples were collected.

Sediment samples should be transported to the laboratory in an insulated chest. Samples should be analyzed for grain size distribution (gravel, sand, and silt+clay fractions) and carbonate, organic matter, and organic carbon content. Analytical methods are described by Erftemeijer and Koch (2001). Although these analyses may be conducted in-house, in most cases a contract laboratory will be used. Samples may be stored at 2°-5° C in a refrigerator for several days or frozen at –20°C for up to one year (see SOP 2.8.3 on storing and analysis of sediment samples).

2.10.7.5 *Voucher Specimen*

Seagrass voucher specimens are collected at each monitoring event for future reference. Collect several complete plants in the vicinity of the center of each cross transect (A-25, B-25, and C-25). Select representative specimens and ensure that you have all the plant parts, including rhizomes and roots. Include plants with reproductive structures when present. Only a small sample of 3-5 complete plants is needed. Place the seagrass sample inside a large, labeled, plastic bag with a small amount of seawater. Indicate with a check mark on the data sheets that voucher specimens were collected.

The voucher specimen should be pressed as soon as possible after collection. If it is going to be more than two hours before the sample is pressed, it should be rinsed in fresh water and refrigerated to prevent any decomposition. Do not refrigerate longer than two days. Rinse the sample in fresh water before pressing and carefully clean of debris, large epiphytes, or sediment particles. Retain the best two specimens for pressing. Lay out each specimen on an acid-free herbarium card, spreading leaves and roots to make each part of the specimen distinctly visible. Fill out specimen labels with the site information, including park code, site name, cross transect, latitude/longitude, date of collection, depth, percent cover, other species present, collector, and comments. Place another clean sheet of herbarium paper over the specimen and layer within newspaper, blotters, and cardboard ventilators within a standard plant press. Tighten the press and allow specimens to dry in a dry, well ventilated place for a minimum of two weeks. For best results, replace the newspaper after two to three days.

Mount one specimen and label on the herbarium card with archival quality glue and maintain with local park collections for reference. Send the other specimen, with its label, to SeagrassNet for archiving (see SeagrassNet manual for contact information).

2.10.8 *Quadrat Measures*

The following measures of seagrass abundance and shoot morphometry are monitored at 12 permanent locations along each cross transect. The sampling locations were selected randomly and are listed on the appropriate cross-transect data sheets (see attachment). Sampling occurs within 0.25 m^2 (i.e. 50 cm x 50 cm) quadrats that are subdivisible into four equal 0.0625 m^2 (25 cm x 25 cm) subquadrats. The exact sampling locations are determined by placing the sampling quadrats on the beach side of the tape measure with the bottom right corner of the quadrat on the sampling distance. For example, the random location for quadrat 1 on cross-transect A is 2 m along the cross transect tape measure, so the quadrat is positioned from 2 m to 2.5 m on the beach side of the tape.

Note that since sampling occurs on the beach side of the tape, monitoring personnel should always walk on the seaward side of the tape to avoid disturbance to sampling areas. Any unattached algae or large mobile animals should be removed from the quadrats before sampling. For simplicity, the methods below instruct field personnel to record measurements on the cross-transect data sheets. In practice, when using snorkel or scuba gear to monitor submerged quadrats, it is easier to record all the data on a dive slate. In this case a grid of data fields identical to the data sheet should be constructed on a dive slate for field data entry. The quadrat data should then be transferred to the cross-transect data sheets and checked for accuracy as soon after sampling as possible.

2.10.8.1 *Photographs*

Photographs are taken of seagrass quadrats as permanent visual records of seagrass condition. They also can serve to verify field data. The quality of quadrat photos is highly dependent on weather conditions. Under ideal conditions (sunny and calm), all quadrats should be photographed. However, if sampling conditions are extremely overcast or windy and wavy, obtaining informative photographs can be difficult and time consuming. Under these conditions, it is acceptable to photograph only three quadrats per transect. These mandatory photo quadrats are those occurring at the beginning, near the middle, and at the end of each transect, and are identified as "required" on the data sheet (with all other quadrats identified as "optional" for photo purposes).

Photos should be taken either before or considerably after other quadrat sampling is done to avoid turbidity created by walking, swimming, or sampling in the area. It is important to either include a quadrat label in the photo or to keep careful field records relating photo numbers to quadrat locations. Be sure that the camera's internal calendar is set to the correct date so that photo file details include this information. Photographs should be taken from as vertical an angle as possible and include the entire quadrat frame and the tape measure. Use the CPVC 0.25m^2 quadrat for improved frame visibility in the photo. Ideally, photos should be taken either when the seagrasses are exposed at the surface (land camera) or when the seagrasses are submerged (underwater camera). Depending on the tide height, when using an underwater camera it may be impossible to capture the entire quadrat frame in a single vertical photo. In this case, individual vertical photos should be taken of sub-sections of the quadrat clockwise from the lower right (i.e. lower right, lower left, upper left, and upper right sub-sections of the quadrat), and a fifth photo should be taken from an oblique angle to capture the entire quadrat frame. Quadrats can be photographed at intermediate depths using a land camera and a plexiglass viewing frame that is floated on the water over the quadrat. A thin film of water spread on the top of the plexiglass prevents reflection, facilitating high-quality photos of the seagrasses under the viewing frame. Mark the "photograph" box under the appropriate quadrat on the datasheet for all photos that are taken. The photo number can be recorded on the data sheet as well.

2.10.8.2 *Percent cover, Shoot Density, Reproductive Shoots*

Record the percent cover of the seagrass canopy within each quadrat, using a laminated percentage cover photo guide as a reference (provided in the SeagrassNet manual). Record the seagrass species within the "percent cover" data field. SeagrassNet procedures for measuring percent cover account for multiple, co-occurring species found

in tropical seagrass beds; total seagrass cover is recorded, as is the percent cover of individual species. In general, North Atlantic Coastal Parks with extensive seagrass resources support single-species beds (*Zostera marina,* eelgrass*;* or *Ruppia maritima,* widgeon grass) and the data sheet associated with this SOP assumes that single species beds are being monitored. Eelgrass and widgeon grass do co-occur in the southern parks in the region (Fire Island National Seashore and Assateague Island National Seashore). If mixed-species beds are most representative of the seagrass resources at a particular park and the monitoring site includes more than one seagrass species, then percent cover of total seagrasses as well as that of each individual species should be recorded (note that the data sheet would need to be modified accordingly by adding additional rows for "total seagrass" and a second species). In this case, note that the percent covers of individual species must sum to the percent cover recorded for total seagrass.

Shoot density is determined in two ways, depending on the size of the seagrass species. For eelgrass, shoot density is determined by counting all shoots present in a 0.0625 m^2 area (i.e. ¼ of the permanent 0.25 m^2 quadrat) at the lower right corner of the quadrat. Form the density subquadrat by threading the fiberglass dividing rods through the circular guides on the quadrat. Record the shoot count within the "density" data field. For widgeon grass, shoot density is determined from the biomass cores by counting the number of leaf meristems within the core sample. In this case, density data are generated during laboratory processing.

Check the quadrat to determine if reproductive shoots (with flowers or fruits) are present. For eelgrass, count the number of reproductive shoots occurring in the entire quadrat and record this number on the data sheet within the "Flowering Shoots" field. For widgeon grass, count the number of flowering shoots within the biomass core sample during laboratory processing and record this number on the data sheet. Note the area of the sample (field count per ¼ m^2 or lab count in cored area) on the data sheet.

2.10.8.3 *Grazing, Epiphyte Cover, Wasting Index*

The degree of grazing, epiphyte cover, and wasting disease (eelgrass only) present in the quadrat is classified using a visual assessment of ten terminal, vegetative shoots. Wasting disease causes characteristic black lesions that spread longitudinally along eelgrass leaves. Burdick et al. (1993) developed a Wasting Index (WI) based on the relative amount of necrotic tissue on the most infected eelgrass leaves in a sample. We adapted the WI for rapid field assessment by classifying the percentage of infected leaf area into one of three categories: trace (T, 0-1%), low (L, 2-30%), or high (H, 31-100%). We selected the threshold between "low" and "high" categories based on data presented by Burdick et al. (1993) suggesting that as the WI exceeds about 30%, wasting disease in an eelgrass population progresses into a phase of rapid infection and disease spread. A similar, cover-based index for estimating *in situ* epiphyte biomass in seagrass beds has been proposed (Miller 1995), although ecologically relevant thresholds have not been defined. We adopted the same T, L, and H classification scheme for assessing grazing and epiphyte cover for sampling convenience.

Examine the most infected leaf of each of ten terminal shoots and classify the percentage of infected leaf area as trace, low, or high as described above. Burdick et al. (1993) provide a visual guide to different percentages of infected leaf area. Keep a mental tally

of the number of leaves falling into T, L, and H categories. Record the overall average category in the "Wasting Index" field on the data sheet. This value is not a true mean of continuous data, but rather is used as an objective measure representing a compromise between field efficiency and quantitative assessment. Measure and record the degree of epiphyte cover by similarly classifying epiphyte coverage of the most epiphytized leaf, and grazing by classifying the degree of grazing evidence (scraped epidermis, bites out of leaf margins, clipped leaf tips) over the entire shoot, for each of ten terminal shoots. With practice, WI, epiphyte cover, and grazing can be assessed simultaneously in a short amount of time per quadrat.

2.10.8.4 *Canopy Height*

Seagrass canopy height is measured by grabbing a clump of plants rooted within the quadrat, extending the leaves (without uprooting the plants) to their maximum height, ignoring the tallest 20% of the leaves, and measuring the distance from the sediment to the top of the remaining 80% of the leaves. Use this technique to measure the canopy height in four locations within the quadrat (one clump of plants in the middle of each quarter of the quadrat); record the average canopy height on the data sheet.

2.10.8.5 *Seagrass Biomass*

2.10.8.5.1 Field Collection

If percent cover of a quadrat is zero, no biomass core is taken. For all quadrats with seagrass present, select an area for sampling that is 0.5 – 1.0 m landward of the quadrat and is of the same percent cover as inside the quadrat. Take a sample using a plastic (0.0035 m^2) or stainless steel (0.0037 m^2) corer, depending on the hardness of the substrate. Place the corer over the area to be sampled, ensuring that all of the leaves originating inside the area to be sampled are inside the corer. Rest the corer on the substrate and run your finger around the perimeter; remove leaves from the corer that are rooted outside the perimeter, and insert any remaining leaves into the corer that are rooted inside the perimeter. It is very important to position the plants inside the corer so that they are not cut during sampling. Push the sharpened edge of the corer vertically into the substrate to a depth of about 10 cm below the depth of the seagrass rhizomes. For eelgrass in the NACP, a core depth of 15 cm is adequate to ensure collection of all root material; widgeon grass rhizomes lie directly beneath the surface, so a core depth of 10-11 cm is sufficient. Place a cap or stopper on the top end of the corer and gently pull it out of the substrate. Seal the bottom of the corer with a cap or your hand as it is removed from the sediment. Empty the entire contents of the corer into a nylon mesh bag labeled with the quadrat number. Rinse the sample gently to remove as much sediment as possible. Record that a biomass sample was taken and the corer size on the data sheet. Place all samples from a single cross transect into a large mesh bag labeled with the cross-transect name. Samples should be transported to the laboratory in an insulated chest.

2.10.8.5.2 Laboratory Procedure

Samples should be processed as soon after collection as possible, ideally on the same day. If samples cannot be processed immediately, rinse the samples in fresh water and store in

a refrigerator for up to two days. All samples should be rinsed in fresh water to remove salt before processing. Empty the sample into a sorting tray and remove all the intact shoots and all the live below ground material; loose, unattached leaves are assumed to have been included in the corer inadvertently and are not retained in the sample, but all living roots and rhizomes are retained. Clean the retained material of any remaining sediment by rinsing in fresh water, and scrape epiphytes off the leaves using a glass microscope slide. Count the number of leaf meristems (the base of the shoot where the leaves attach to the rhizome) in the sample and record on the data sheet in the "Number of meristems in biomass core" field. Separate the shoots into the following sample components for biomass determination: 1) leaves, including all intact material connected to a rhizome (green leaves as well as any dead attached leaves); 2) sheaths, including only living sheaths attached to rhizomes without blades still attached (i.e., sheaths with blades attached are included in the leaf component, and black, dead sheaths without blades attached are excluded); 3) roots and rhizomes, including all the living rhizomes (whitish to brown in color) and roots. Place each clean biomass component in separate paper bags labeled with the sample date, site abbreviation, cross-transect letter, quadrat number, and plant part. Dry the samples in a drying oven at 60^{o} C for 48 hours or until completely dry. Cool the dried samples in a desiccator and weigh on an electronic balance in grams to an accuracy of three decimal places. Record the sample identification and weight on a data sheet or Excel spreadsheet.

2.10.9 *Post-sampling Procedures*

2.10.9.1 *Downloading Data from the Light Loggers*

- Open the BoxCar® software package on your computer.
- Connect the first Hobo LI light logger to the computer using the interface cable provided with the software. Note that the cable is equipped with a 9-pin RS-232 female connector for direct connection to the computer's serial port. If your computer has only USB ports then a USB Serial adapter (available from Onset®) will be necessary to connect the Hobo LI logger to the computer.
- Select "Readout" from the Logger menu. A dialog box will appear that says "Connecting..." and then "HOBO found"
- The "Offload" dialog box will appear and signal that the data are downloading
- The "Disconnect Logger" dialog box will appear. Unplug the logger from the PC interface cable and select "OK"
- The "Save As" dialog box will appear. Select the subdirectory (folder) in which to save the data file and enter a file name. The file name should include should include the 4-letter park unit code, site name, station label (A, C, or "Air"), the data type (light or temp) and monitoring period. Use underscores to separate codes for legibility in the file name, e.g. "CACO_DH_A_light_July2004"
- Select "Save" to save the data in the file/folder identified as a *.dtf file for viewing within BoxCar software.

- Export the data to an Excel file by selecting "Export" and "Microsoft Excel Spreadsheet" on the File menu for additional analysis. Use the same naming convention for the data file.
- Repeat this process for the remaining two light loggers.
- Record the date, time, and file names of downloaded data on the Light and Temperature Sensor Deployment Log.

2.10.9.2 *Downloading Data from the Temperature Loggers*

The following instructions pertain to Onset Stowaway TidBiT® temperature loggers. Detailed instructions for downloading temperature data from iButton® temperature loggers are provided in the SeagrassNet manual.

- Open the BoxCar® software package on your computer.
- Connect the first TidbiT® onto the TidbiT® coupler attached to the Optic Base Station.
- Select "Readout" from the Logger menu. A dialog box will appear that says "Connecting..." and then "Stowaway found"
- The "Offload" dialog box will appear and signal that the data are downloading
- The "Disconnect Logger" dialog box will appear. Unplug the logger from the PC interface cable and select "OK"
- The "Save As" dialog box will appear. Select the subdirectory (folder) in which to save the data file and enter a file name. The file name should include should include the 4-letter park unit code, site name, station label (A or C), data type (light or temp) and monitoring period. Use underscores to separate codes for legibility in the file name, e.g. "CACO_DH_A_temp_July04-05"
- Select "Save" to save the data in the file/folder identified as a *.dtf file for viewing within BoxCar software.
- Export the data to an Excel file by selecting "Export" and "Microsoft Excel Spreadsheet" on the File menu for additional analysis. Use the same naming convention for the data file.
- Repeat this process for the remaining temperature logger.
- Record the date, time, and file names of downloaded data on the Light and Temperature Sensor Deployment Log.

2.10.9.3 *Quadrat Photograph Management*

Digital quadrat photographs should be downloaded as soon as possible after they have been taken to ensure accurate photo identification. If a quadrat label was not included in the photos, then all photos must be related to quadrats using field notes. Name each photo file with the 4-letter park unit code, site name, cross-transect, quadrat, and subquadrat/view if applicable (i.e. in instances where water depth did not allow vertical

photographs of the entire quadrat, label vertical subquadrat photos "a" – "d" in the order taken and label the oblique full quadrat photo "e"). E.g., CACO_PB_A1, CACO_DH_C2a. File properties should include the photo date, but the files should also be organized into folders named by site and date.

2.10.9.4 *Field Equipment Clean Up*

The corrosive properties of salt water and damaging effects of salt crystals can shorten the life of your field gear. Therefore, it is very important to rinse all sampling equipment in fresh water after use. This is also the best time to inventory sampling supplies and equipment and replace any items that will be needed for the next monitoring period.

2.10.10 *References*

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Miller, R. R. and R. W. Virnstein. 1995. Development and use of a photo-index as a tool for monitoring epiphyte biomass on seagrass in the Indian River Lagoon, Florida. Estuarine Research Federation 13th International Conference, November 12-16, Corpus Christi, TX.

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Short, F. T., L. J. McKenzie, R. G. Coles, and J. L. Gaeckle. 2004. SeagrassNet manual for scientific monitoring of seagrass habitat – Western Pacific Edition. University of New Hampshire, USA, QDPI, Northern Fisheries Centre, Australia. 71pp.

2.11 SOP 11 – Water Quality Monitoring Data Reduction

2.11.1 *Spatial Water Quality Surveys*

Data collected by YSI sondes for the spatial component of water quality monitoring is uploaded from the sonde in PC6000 format. This format results in a computer file with a .dat file extension. The default program for this extension is YSI EcoWatch, and the file will not be readily editable, making it ideal for archiving purposes. In order to perform the data reduction steps in this SOP, use the <export> feature in EcoWatch to create a comma-delimited file (.cdf), which can then be imported by most spreadsheet and database programs. To assist in file tracking and management, change only the file extension when converting from one format to another, but do not alter the filename. Also create a text document (.txt) with this same filename for documenting all data reduction steps that are specific to that data file (date rejection). Data collected using LiCor quantum sensors for spatial monitoring of photosynthetically available radiation (PAR) are recorded on field data sheets. For each station sampled during a spatial survey, perform the data checks and data reduction steps described below.

2.11.1.1 *Dissolved Oxygen Calibration Check:*

Check the calibration of the dissolved oxygen sensor before and after each hydrocast. These calibrations are part of the data stream for each station since the sonde starts logging while still in water-saturated air. The in-air data (identified using the depth channel) at the beginning and end of each hydrocast should read between 97-103%. If this is not the case, then proceed as follows:

i. If the pre-deployment DO %saturation fails this test, then omit the entire hydrocast from the data analysis. This occurrence should be very rare since the operator is instructed to perform a check in the field and take corrective action if necessary.
ii. If only the post-deployment DO % saturation fails this test, and if bottom water dissolved oxygen concentrations were less than 1 mg/l, or if there is a record of the sonde having hit the substratum, then use only the downcast for data analysis (see section 2.11.1.3 on sulfide interference).
iii. If only the post-deployment DO % saturation fails this test but low dissolved oxygen was not encountered, examine the dissolved oxygen and DO charge channels carefully and use best professional judgment on whether data can be salvaged.

2.11.1.2 *Dissolved Oxygen Membrane Check*

Check the "DO charge" for each reading on both down- and upcasts to see that it falls within the range of 25-75. If it falls outside this range, discard the dissolved oxygen readings (percent saturation and concentration) associated with this failed "DO charge" reading.

2.11.1.3 *Sulfide Interference Check*

The presence of hydrogen sulfide will interfere with the Clarke-type electrode used for dissolved oxygen and make the output "jumpy". This effect is seen when the bottom

water is anoxic and H_2S is present or when the sensor is run into anoxic sediments. Reject faulty data as follows:

i. Check to see if the sonde touched the bottom during the deployment (reported on the Station Data Sheet). If so, and if the dissolved oxygen channels show erratic performance on the upcast, use only the downcast dissolved oxygen data for further analysis.
ii. Check to see if the sonde encountered low dissolved oxygen during the hydrocast. If so, then use only the downcast data for further analysis and use best professional judgment to clean up dissolved oxygen data from the downcast data.

2.11.1.4 *Turbidity Interference Check*

If the sonde touched the bottom during the deployment (reported on the Station Data Sheet), then bottom sediments were likely stirred up. These sediments will interfere with subsequent near-bottom turbidity readings, and may also interfere with chlorophyll-*a* readings. As a crude tool for removing these artifacts, reject all turbidity and chlorophyll data from the upcast. It should, however, be feasible to salvage some of the upcast turbidity and chlorophyll data through a careful examination of hydrocast data.

2.11.1.5 *Sensor Performance Specification Check*

Reject data that fall outside the design specification range for each sensor. These specifications are currently as follows:

Temperature	-5 to 45 ˚C
Conductivity	0 to 100 mS/cm
Dissolved Oxygen, % saturation	0 to 500% air saturation
Dissolved Oxygen, mg/l	0 to 50 mg/l
Dissolved Oxygen charge	25-75
Salinity	0 to 70 ppt
Turbidity	0 to 1000 NTU
Chlorophyll	0 to 400 µg/l

2.11.1.6 *Calculate the Average Station Location and Time*

Average all of the latitude, longitude, and time data from both down and up-casts to yield the average location of the hydrocast. Be sure not to use data collected during the in-air warm-up and post deployment checks since the vessel may be underway during these periods.

2.11.1.7 *Depth Binning of Water Quality Data*

Sort data into 0.5 m depth bins, coded by upcast or downcast. Exclude data collected prior to the start of each profile (during the in-water equilibration period), as well as data collected in air during the oxygen calibration check. Be particularly careful also to exclude data collected during operation of the optical wipers, which is supposed to happen during the equilibration period.

i. Calculate the number of readings and the average value for each variable for each depth bin on the downcast, the upcast, and the average of these two.
ii. For each station, create a data table with the following fields:
    a. Bin depth (upper limit in m)
    b. Downcast or upcast
    c. Number of samples per bin
    d. Bin-averaged temperature (ºC)
    e. Bin-averaged salinity (psu)
    f. Bin-averaged dissolved oxygen concentration (mg l^{-1})
    g. Bin-averaged dissolved oxygen saturation (% sat)
    h. Bin-averaged Turbidity (NTU)
    i. Bin-averaged chlorophyll-a (uncorrected µg l^{-1})
    j. Bin-averaged chlorophyll-a (corrected µg l^{-1})

Note that fields a-i are derived directly from the station data file. Derivation of field j in described below.

2.11.1.8 *Chlorophyll-a Post-Calibration*

The bin-averaged chlorophyll-a (corrected µg l^{-1}) must be post-calculated using the laboratory analysis of chlorophyll-*a* extracted from discrete grab samples. Perform the following steps to calculate the correction.

i. For the surface (0.0-0.5m) depth bin, calculate the average uncorrected chlorophyll-*a* concentration (average of the downcast and upcast means for the surface bin).
ii. Perform a linear least squares regression of this uncorrected chlorophyll-*a* data (dependent variable) against chlorophyll-*a* from extractive analysis on discrete grab samples (independent variable).
iii. Use the equation for this regression to correct chlorophyll-*a* and fill in the last data field (above).

2.11.1.9 *Processing of Light Data*

Use the LiCor light data to calculate the attenuation coefficient of downwelling PAR (K_d).

i. K_d should be first calculated as the slope of the least squares regression of $\ln(I_z/I_{air})$ against depth in meters, where I_z is the irradiance at depth z, and I_{air} is the irradiance in air. Units of K_d are m^{-1}.
ii. I_z/I_{air} is recorded on the Station Data Sheet under the column labeled "Channel M2 - Underwater Irradiance- % of I_{air}." (Note: the data in this column are actually I_z/I_{air} x 100, but this makes no difference.)
iii. Calculate the coefficient of determination (r-squared) for the fit of this least square linear regression.
    a. If the r-squared is 0.95 or greater, report the slope of this line as K_d for this site. This method accounts for variation in ambient irradiance caused by changes in cloud cover.
    b. If the r-squared is less than 0.95, then calculate K_d as before, but substituting I_z for the ratio I_z/I_{air}. This data is labeled "underwater irradiance – PPFD" on the Station Data Sheet. If the r-squared for the new

regression is 0.95 or greater, then use this new slope as K_d for the site. This method may yield better results if the readings in air ("deck" sensor) were faulty, but does not account for changes in cloud cover.

c. If neither approach yields a coefficient of determination ≥ 0.95, then select the slope of the regression with the higher r-squared, provided it is ≥ 0.9. If this condition cannot be met, then use best professional judgment to determine whether a representative K_d can be calculated by eliminating outliers in the data or including data that had originally been struck through by the field technician.

2.11.2 *Continuous Water Quality Monitoring*

The purpose of this section of the SOP is to provide quality assurance guidelines for determining what portions of the data should be included in the final record and used for analysis. Data rejection criteria are based upon recommendations and guidance from the sonde manufacturer, YSI, Inc. The following sections borrow liberally from data analysis guidance developed by YSI for the NOAA National Estuarine Research Reserve System for its System-Wide Monitoring Program (YSI 2000). By following closely with the NOAA NERR protocols, NPS will benefit from their years of experience in continuous estuarine monitoring as well as from the ability to interpret NPS data within a larger spatial context.

Data reduction for continuous monitoring is complicated by the fact that the sensors are deployed for an extended period between initial calibration and post-deployment calibration check. During this interval, sensor response may be corrupted by a number of factors including drift, biofouling, and catastrophic damage by organisms or extreme environmental conditions. The SOP for Continuous Water Quality Monitoring is designed to maximize the collection of high-quality data that are accurate and representative of environmental conditions during the index period. Drift and biofouling effects can usually be corrected using data from the post-deployment calibration check and the weekly quality assurance visits to the logging station, which are made during each of the spatial survey cruises. Proper application of this SOP will assist in making data corrections where they are possible, and in culling out any remaining poor-quality data from the official record.

Data collected by YSI sondes for the continuous components of water quality monitoring are uploaded from the sonde in PC6000 format. This format results in a computer file with a .dat file extension. The default program for this extension is YSI EcoWatch, and the file will not be readily editable, which makes it ideal for archiving purposes. In order to perform the data reduction steps in this SOP, use the <export> feature in EcoWatch to create a comma-delimited file (.cdf), which can then be imported by most spreadsheet and database programs. To assist in file tracking and management, change only the file extension when converting from one format to another, but do not alter the filename. Also create a text document (.txt) with this same filename for documenting all data reduction steps that are specific to that data file (data rejection).

2.11.2.1 *Absolute Data Rejection*

Absolute data rejection is a means of removing erroneous values from the data record because they violate basic principles of sensor behavior and/or proper sonde deployment. Because it is based upon basic principles, absolute data rejection can be accomplished using automated computer scripts with conditional arguments. There are at least two cases in which absolute data rejection must be invoked. The first of these is when the recorded value for any sensor falls outside its performance specifications. Environmental specifications for each sensor are listed in section 2.11.1.5 above. Any data that fall outside the specification intervals should be removed from the official record.

The second case is when one or more of the sensors is believed to have come out of the water due to unexpected tidal fluctuations or improper deployment. In brackish and marine systems, this occurrence is indicated by anomalously low readings from the specific conductance sensor. The conductivity sensor is the highest/shallowest of the sensors on the YSI 6600EDS and will be the first to be exposed to air. Since it is impossible to determine which other sensors were also out of water, all data from these out-of-water events should be removed from the record. Note that the upper PAR sensor from a customized YSI 6600EDS will actually come out of the water before the conductivity probe, but this is difficult to discern using programming scripts and does not affect the other probes.

2.11.2.2 *Discretionary Data Rejection, Drift Correction and Data Reduction.*

This portion of the SOP is not easily automated using logic statements, and must carried out manually under the supervision of a program manager with experience in continuous water quality monitoring and knowledge of the YSI sonde and its application in this Protocol. Ideally, these steps should take place as soon as possible after sonde recovery so that the details of the deployment are fresh in the minds of the field personnel, and so that corrective action can be attempted for any subsequent deployments. A rapid evaluation for any gross problems with the data record can be made using the graphing features of YSI's EcoWatch software. Examine the output from each sensor individually for any discontinuity in the data, which generally indicates catastrophic failure during the deployment. To check for sensor drift, examine the logged data in comparison with the discrete measurement that were made as part of the weekly spatial survey. Sensor-specific details for data rejection and drift correction are provided in the following sections.

2.11.2.2.1 Temperature

The temperature sensor on the YSI 6560 temperature and conductivity probe is very robust and is unlikely to fail. Rare failure has been observed, however, and is most often associated with an irreversible malfunction due to leakage of the sensor housing. Incorrect temperature data are signaled by a clear point of temperature discontinuity followed by unreasonable and erratic values or unreasonable drift. If clearly incorrect temperature data are observed, all data from that point on should be removed from the official record. Since all the other sensors are temperature compensated using values from the 6560 thermistor, ALL data following failure of a temperature probe are suspect and should be removed from the official record.

2.11.2.2.2 Conductivity

Like the thermistor on the YSI 6560 temperature and conductivity probe, the conductivity cell is very robust and rarely shows catastrophic failure. If an error occurs, it is usually a drift of the output due to biofouling within the water ports of the conductivity cell. This results in a change in the effective volume of the cell, which, in turn, results in drift of the output. The post-deployment calibration check will show whether such a drift has occurred. Cleaning of the sensor ports should resolve the issue. In these cases, at the discretion of the program manager, a linear compensation may be applied to the data using quality assurance data from the weekly surveys and from the post-deployment calibration check.

In the unlikely event of catastrophic sensor failure, a sharp discontinuity will be evident in the output, and all subsequent data should be removed from the official record.

2.11.2.2.3 Dissolved Oxygen

The YSI 6562 dissolved oxygen probe is susceptible to both drift and catastrophic failure, although drift is less significant a problem when the sensor is mounted in the YSI 6600EDS. This is because drift usually results from biofouling over the probe membrane over the course of the deployment. The post-deployment calibration check will show whether such a drift has occurred. At the discretion of the program manager, a linear compensation may be applied to the data using quality assurance data from the weekly surveys and from the post-deployment calibration check.

Catastrophic failure of the dissolved oxygen probe is more common, and is usually the result of a puncture in the membrane, either by debris or organisms, or from an improperly installed membrane. Under these scenarios, output is generally characterized by a large discontinuity. In most cases readings become unreasonably high very quickly and then either become very noisy or drift around. The likely cause of this behavior is "crosstalk" through the membrane hole caused by electrical continuity between the dissolved oxygen and conductivity sensors through the brackish estuarine water. All data after the initial discontinuity should be removed from the record, regardless of later probe behavior. After this type of catastrophic failure, the probe surface should be reconditioned before it is put back in service. Follow guidance in the YSI 6-Series Environmental Monitoring Systems Manual. Occasionally the DO probe will fail due to structural failure of the Clark-type electrodes. The symptoms are similar, and are accompanied by high DO-charge values. All data after the initial discontinuity should be removed from the official record.

2.11.2.2.4 Turbidity and Chlorophyll

YSI's optical turbidity and chlorophyll probes are very stable over time and not prone to drift. Consequently, there should be no need to manually adjust the data using linear compensation. When the turbidity and chlorophyll optical sensors produce erroneous data, it is usually due to physical obstructions in the measured volume of water. These obstructions could be from fouling organisms, debris, or wiper pads that fail to park in their proper locations. These types of interference are often characterized by very large negative values in the data record. These negative values should be removed from the

record. Since they are outside the design specifications for the sensors, they should be automatically removed by the filters used for absolute data rejection (see section 2.11.2.1). Since it is possible for these physical obstructions to come and go over the course of a deployment, and since they generally cause no lasting effect on sensor response, it is NOT necessary to remove all subsequent data after one of these out-of-bounds data spikes has occurred. The program manager may use professional judgment in filtering out other optical data that are clearly inconsistent with the overall data record, such as occasional very-high positive peaks or high-frequency and very-high-amplitude noisy data (in which case the entire interval should be removed rather than just the peaks).

2.11.2.2.5 Photosynthetically Available Radiation

The first step to assuring the quality of continuous PAR data is to compare pre and post deployment calibration data for each of the two LiCor 192SA sensors. These sensors are vulnerable to catastrophic failure from water vapor that slowly diffuses through the acrylic optical diffuser. If either sensor shows a dramatic drift (>15%), then suspect catastrophic sensor failure. Through careful examination of the data record, it may be possible to identify a point when failure occurred. Remove from the final record all data after this point for the affected sensory only. Although PAR attenuation coefficients cannot be calculated during periods with only one functional sensor, it still provides a record of the underwater light field during the index period.

In some instances, catastrophic failure is not abrupt, making it more difficult to assign a cutoff point. The PAR data collected for quality assurance during the weekly spatial survey cruises are useful for identifying the timing of sensor failure, and also for applying a linear correction when PAR sensors have drifted less than 15%.

The principal metric of interest for this protocol is the attenuation coefficient of PAR, K_d. To calculate K_d, only a subset of the PAR data is used. This subset is all PAR data collected within 3 hours of local apparent noon. This is a slight extension to the interval recommended by Carruthers et al. (2001), but will still generate very good data while allowing comparison with data collected in the spatial survey. For all anticipated applications of the protocol, this time interval will run from 10:00 hrs to 16:00 hrs Eastern Daylight Savings Time. Include the data from the 10:00 AM logging event even though it technically represents the last minute of the preceding hour.

Create a new data column for K_d, and populate it (for the above subset) using:

$$K_d = \frac{\ln(I_z / I_0)}{-z}$$

where I_z is the reading from the lower PAR sensor, I_0 is the reading from the upper PAR sensor, and z is the separation between them in meters (0.5 m). Units for K_d are m^{-1}. Examine the logged K_d values against those collected for quality assurance purposed at the time of deployment, retrieval, and during each of the weekly spatial surveys. At the program manager's discretion, a correction factor and linear compensation for drift can be applied to the logged data using the quality-assurance data and the post-deployment calibration check.

2.11.2.3 *Chlorophyll-a Post-Correction*

The chlorophyll sensor on the YSI 6600EDS is calibrated using a Rhodamine WT dye standard. Used alone, this calibration method will report consistently from deployment to deployment, but will not necessarily provide an accurate representation of actual pigment concentrations. More accurate reporting of chlorophyll-*a* is achieved by calibrating these *in situ* fluorometic values against chlorophyll-*a* data from extractive analyses on discrete grab samples. For each YSI sonde deployment, these calibration samples are collected at the beginning, end, and intermittently throughout each deployment. (see the SOP on Chlorophyll-*a* Sampling and Analysis). Follow these steps to post-correct against extracted chlorophyll.

i. Identify all the logged dye-calibrated chlorophyll samples that coincided with the collection of a discrete grab sample
ii. Perform a linear least squares regression of the dye-calibrated chlorophyll-*a* data (dependent variable) against chlorophyll-*a* from extractive analysis on discrete grab samples (independent variable).
iii. Use the equation for this regression to correct chlorophyll-*a* and create a new data field for corrected chlorophyll (retaining the uncorrected data as well).

Should the correlation coefficient for the regression be poor, or should the range of the correction data under grossly under-represent field concentrations, then the program manager should use best professional judgment in deciding whether and how to apply the correction.

2.11.3 *References*

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2.12 SOP 12 – Data Management

2.13 SOP 13 – Data Analysis and Annual Reporting

2.13.1 *Water Quality and Sediment Organic Carbon Reporting*

There are three components for analysis and reporting of sediment and water quality monitoring data. These are the area-weighted spatial survey, trend station, and continuous monitoring components. For the area-weighted component of the protocol, estuarine waters are treated as a continuous resource, and sampling stations are selected randomly from within hexagons of a randomly overlaid grid (see SOP 1). Except for a small subset of the hexagons assigned with trend stations, sampling is re-randomized each year. This reduces the bias associated with deliberate station positioning. It also allows us to report area-weighted average values of condition for each estuary, and the proportion of each estuary falling into specific categories of condition. The methods for this aspect of data analysis and reporting are addressed below in section 2.13.1.1. As a starting point for reporting of water quality data from the spatial survey, data reduction should be performed so that the metrics listed in Table 17 are reported for each hexagon spatial element for each park.

Table 17. Data to be included in the data report in tabular format and organized by park, stratum, and spatial element number (hexagon ID). Note that all variables are reported annually except for sediment organic carbon, which is only sampled every fifth year.

	Single value for station	Volume-weighted water column average & stdev	Mean & stdev from surface 0.5 m depth bin	Mean & stdev from deepest 0.5 m bin
Dissolved oxygen (mg l^{-1})		X	X	X
Dissolved oxygen (% saturation)		X	X	X
Chlorophyll-a (µg l^{-1})		X	X	X
Turbidity (NTU)		X	X	X
PAR Attenuation (K_d m^{-1})	X			
Temperature		X	X	X
Salinity		X	X	X
Sediment organic carbon (%)	X			

The second component of analysis and reporting is that associated with trend stations. For NCBN parks without pre-existing monitoring programs, 20% (6) of the hexagons are assigned trend stations. Trend stations are initially positioned randomly within their hexagons, but are not re-randomized each year. This eliminates spatial variability in the analysis of data from within a hexagon, and as the name implies, is useful for identifying inter-annual variability and trends. Also, trend stations are visited four times during the index period. This allows for the evaluation of variability within the monitoring interval. Parks with existing monitoring programs are evaluated individually to see if existing station can be adopted as trend stations for this protocol (see SOP 1). For those parks, the number of trend stations exceeds 20% in some cases. The methods for analysis and reporting of trend station data are described in section 2.13.1.2 below.

The final component of data analysis is for the continuous monitoring stations. Since most parks have only one continuous station, this data is not spatially representative. Instead, it is used to examine temporal variability during the index period. Section 2.13.1.3 describes the reporting requirement for these data.

In addition to each of the above analysis and reporting components,

2.13.1.1 *Area-Weighted Spatial Data*

Area-weighted water quality and benthic condition within each estuary is reported in two ways: 1) the mean condition and 2) the percent of the estuarine area exceeding threshold values for selected parameters. Estimation procedures for mean parameter values and variance are weighted by the estuarine area within the tessellated hexagon from which each sample is collected (Cochran 1997). CACO and ACAD are special cases because they use multiple strata designs (SOP 1, Stevens 1997). For these parks, each stratum is evaluated individually and then also combined for a park-wide synopsis.

Note that, for this protocol, at least 6 of the hexagons from each park have been assigned as trend stations. Not only are these stations sampled every year, they are also sampled each week during the index period. For this component of the analysis, however, data are only used from the first date within the index period that a trend station is successfully sampled. This will help to prevent biasing the analysis and averaging out extreme events. The subsequent repeat visits to trend stations are used for examining changes within the index period (see section 2.13.1.2).

Special consideration is necessary for the few parks with existing monitoring programs where multiple trend stations are positioned within a single hexagon. In these cases, area-weighting of data must be modified to apportion the estuarine area among the multiple stations. ASIS, for example, has three existing monitoring stations within hexagon 27. Two of these are considered representative and are allowable as trend stations for NCBN monitoring. These fall on opposite sides of a peninsula that bisects the hexagon, so each should be assigned spatial weighting and inclusion probabilities based only upon the estuarine area on their side of the peninsula. Where no such physical barrier exists, the weighting should be divided evenly between the two stations unless professional judgment dictates otherwise.

Area-weighted mean value of any metric are calculated use the following equation (USEPA 2002 and Diaz-Ramos et al. 1996).

$$\bar{y} = \frac{1}{A}\sum_{i=1}^{n}\frac{y_i}{\prod_i} \qquad \text{Equation 1}$$

where:

$\bar{y}$ = the area-weighted mean
Π_i = the inclusion probability for a station (1/ estuarine area within hexagon i)
A = the total area of sampled estuarine water in the stratum
y_i = the variable of interest measured in hexagon i

n = the number of hexagons sampled in the stratum

For most of the North Atlantic Coastal Parks, all estuarine resources fall within a single park stratum. For CACO and ACAD, however, the mean condition from multiple strata must be combined to generate a value for the whole park. This will be the area-weighted average from the individual strata.

$$Y = \sum_{i=1}^{m} w_i \bar{y} \qquad \text{Equation 2}$$

where:

Y = the estimated mean condition for the combined strata,
w_i = relative area of stratum i to the total park area.
m = the number of strata

The Horvitz-Thompson estimator is commonly used to calculate sample variance for this type of design (Cochran 1997); however, Stevens and Olsen (2003) have developed a local neighborhood variance estimator specifically targeted toward accommodating the nature of this design. Either may be applied here, but the latter yields notably smaller variance, particularly when there are strong spatial patterns associated with the data.

Average park-wide condition shall be reported for each of the variables listed in Table 18. These data are to be included in the annual data report in tabular and graphical format. For parks with multiple strata, the values are reported for each stratum, and also combined into a park-wide estimate using Equation 2.

Table 18. Average condition of the estuary for selected area-weighted variables. These data are to be included in the annual data report in tabular and graphical format. Station values are weighted by the area of estuarine resource within the respective hexagons. For parks with multiple strata, these values are reported for each stratum, and then also combined into a park-wide estimate. Water column data are volume weighted by first grouping discrete sampled into 0.5 m depth bins and then averaging bins.

	volume -weighted water column average	surface layer (surface 0.5 m depth bin)	bottom water (deepest 0.5 m depth bin)	Single measure per station
Dissolved oxygen (mg l^{-1})	X	X	X	
Dissolved oxygen (% saturation)	X	X	X	
Chlorophyll-a (µg l^{-1})	X	X	X	
Turbidity (NTU)	X	X	X	
Temperature (ºC)	X	X	X	
Salinity (psu)	X	X	X	
PAR Attenuation (K_d m^{-1})				X
Sediment Organic Carbon (%)				X

The Estuarine Monitoring Database includes automated routines for generating the volume-weighted averages, as well as the surface, bottom water values. From the *Main Menu*, select *Analysis and Export*, then select *Summary Reports* (Figure 19). From the list of available reports, highlight *Spatial Survey WQ Data - weighted averages*. Click *Preview* to view the report or *Print* to print it.

To export a digital version of this data for direct inclusion in a text document of for use in a spreadsheet or other program, select *Analysis and Export* from the *Main Menu*, then select *Export Data to Excel*. From the list of available reports, highlight *Spatial Survey WQ Data - weighted averages*. Click *Preview* to view the table or *Export* to save it to an Excel formatted file.

Similar reports and output are available for the area-weighted means compared by park and year. Select *Spatial Survey WQ Data – averaged by park*, from the same menus above to report these data.

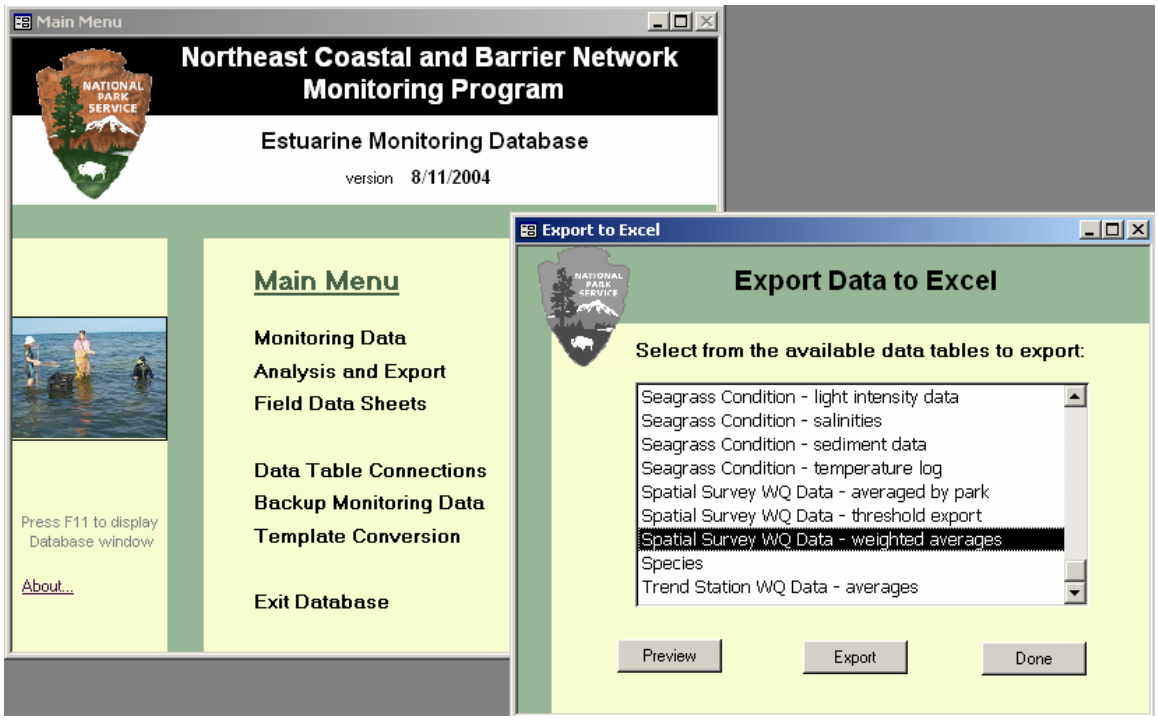

Figure 19. Estuarine Monitoring Database menus for previewing and exporting data.

The second approach to reporting of spatial data is to use rating criteria. For this, NCBN has adopted critical threshold values (Table 19) that have been accepted by the US EPA for national coastal condition reporting (USEPA 2001, 2004). These are also largely consistent with the thresholds used for the NOAA National Estuarine Eutrophication Assessment (Bricker et al. 1999). The only change to these thresholds is that water clarity is expressed as K_d (m^{-1}) rather than as an index. Cutoff values of K_d have been calculated from the EPA criteria for turbidity using the thresholds for areas with significant SAV resources. For NCBN reporting on these indicators, the proportion of the resource area that falls into each of four quality categories (good, fair, poor, or missing) shall be calculated for each park (and where applicable, stratum). The fourth ("missing")

category is used when there is missing data for any of the spatial elements within a park. Equations for making these calculations are given below; however, MicroSoft Excel worksheet macros have been developed for this purpose at the US EPA at the Narragansett Atlantic Ecology Division Laboratory in Narragansett, RI (Current contacts are John Kiddon kiddon.john@epamail.epa.gov and Hal Walker walker.henry@epamail.epa.gov). Example charts for reporting of proportion data are shown in Figure 20. Cumulative frequency distribution plots are useful for interpreting data grouped by park or stratum.

Table 19 Critical values used in establishing the portion of the estuary (or stratum) that falls within a given quality category. These are based upon the threshold values that are in use by both the US EPA National Coastal Assessment and the NOAA National Eutrophication Assessment (Table 19; USEPA 2001, 2002, 2004, and Bricker et al. 1999).

	Good	Fair	Poor
Chlorophyll-*a* (surface0.5 m depth bin)	< 5 µg /l	5-20 µg /l	> 20 µg /l
Dissolved Oxygen (bottom 0.5 m depth bin)	> 5 ppm	2-5 ppm	< 2 ppm
Water Clarity K_d (SAV standard)	< 0.92 m^{-1}	0.92-1.61 m^{-1}	> 1.61 m^{-1}
Sediment Total Organic Carbon (TOC)	< 2%	2-5 %	> 5%

The proportion of the area of a resource area meeting a prescribed threshold is:

$$\hat{P}_z = \frac{1}{A}\sum_{i=1}^{n}\frac{y_i}{\Pi_i} \qquad \text{Equation 3}$$

where

$\hat{P}_z$ = the estimated proportion of the area at or below a response value of z
y_i = 1 if the response value is less than or equal to z, and otherwise is 0
Π_i = the inclusion probability for a station (1/ estuarine area within hexagon i)
A = the total area of sampled estuarine water in the stratum
n = the number of hexagons sampled in the stratum

When combining strata for CACO and ACAD, the park-wide proportions are calculated as:

$$\hat{U} = \sum_{i=1}^{m} w_i \hat{P}_i \qquad \text{Equation 4}$$

where

$\hat{U}$ = the estimated proportion area for the combined strata

$\hat{P}_i$ = the estimated proportion area for the stratum *i*
w_i = relative area of stratum *i* to the total
m = the number of strata

The Microsoft Excel macros developed and provided by EPA currently use the binomial approximation for calculating variance on proportion data. Jackknife methods may provide more realistic estimates, but have not as yet been coded into the programming (John Kiddon personal communication).

The Estuarine Monitoring Database includes automated routines to export these data in Excel format to be analyzed with the EPA macros. Select *Analysis and Export* from the *Main Menu*, and then select *Export Data to Excel*. From the list of available reports, highlight *Spatial Survey WQ Data – threshold export*. Click *Preview* to view the table or *Export* to save it to an Excel formatted file.

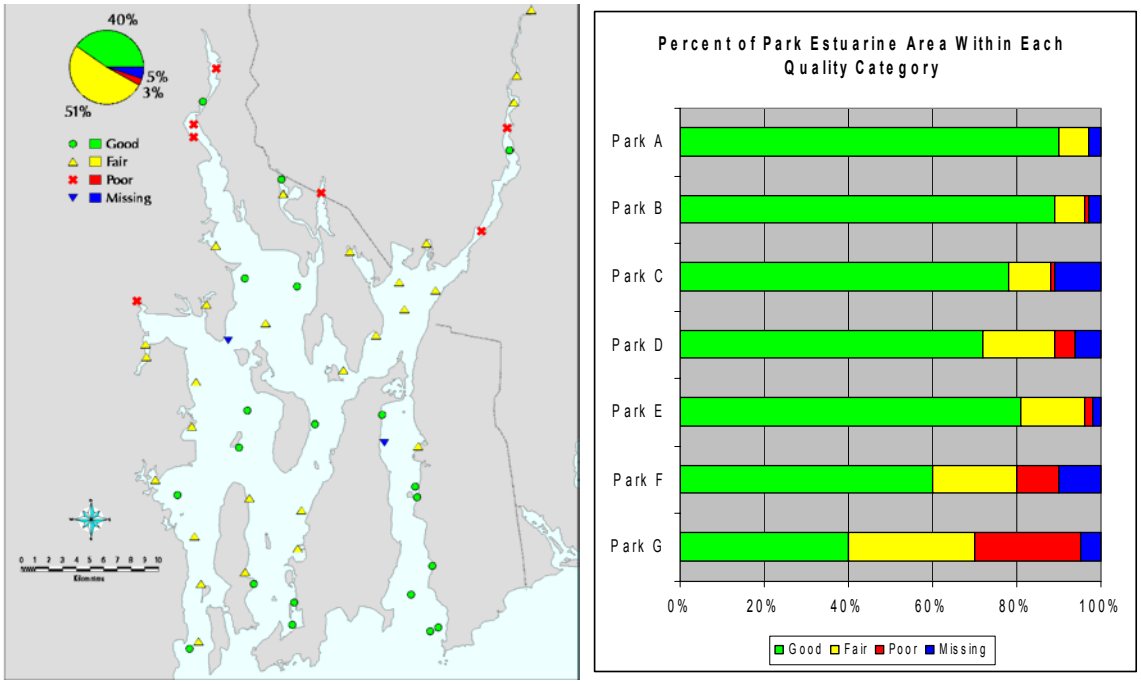

Figure 20. Example graphics for reporting of area-weighted proportion data (figure A courtesy John Kiddon, US EPA).

2.13.1.2 *Trend Station Data*

Data from the trend stations serve two purposes; 1) detecting interannual change (trend analysis) and 2) evaluating variability of the indicator metrics in time (through the index period) and through space (throughout the estuary). Because their positions are not re-randomized each year, spatial variability is reduced. This makes them useful for detecting interannual change and long-term trends. By keeping the number of trend stations low (20%) they are able to serve double duty for estimating area-weighted average condition (section 2.13.1.1) without adding unnecessary bias to those estimates.

And because trend stations are measured four times during each index period, we are able to interpret results of the weekly spatial survey within the context of temporal variability within the index period as well as spatial variability throughout the estuary.

For analysis and reporting purposes, data should be evaluated by individual station. Means and standard errors for the index period should be calculated for each of the trend stations; and this data should be examined for patterns associated with location in the estuary, as well as for trends with past year data. While averaging through time is appropriate, averaging across stations is to be avoided because equal weighting is not a reasonable assumption. That is the role of the probability survey and the area-weighted data analysis (section 2.13.1.1).

The means and standard errors for the trend stations are available as both Access reports and as Excel exports. From the *Main Menu*, select *Analysis and Export*, then select either *Summary Reports* or *Export to Excel*. From the list of available reports, highlight *Trend Station WQ Data - averages*. Click *Preview* to view the report, *Print* to print it, or *Export* to export the data.

2.13.1.3 *Continuous (Index Period) Water Quality Data*

The role of the continuous monitoring data is similar to that of the trend data except that temporal resolution is further emphasized - displacing any ability to resolve spatial patterns. Estuaries are highly pulsed systems, and even the weekly sampling at trend stations may reasonably be expected to miss ecologically important events such as phytoplankton blooms, or episodes of low dissolved oxygen and high turbidity. All spatial data from the weekly survey cruises should be evaluated within the context of the continuous record for that period. This will improve interpretation and also allow for ongoing evaluation of the timing and duration of the index period used for all NCBN estuarine monitoring.

Although not spatially representative, data from the logging station can also be used to track inter-annual changes. Because there is no spatial replication, we can only interpret results within the context of that one station. Temporal replication, on the other hand, is very good. Each logging station is sampled 96 times per day (as many as 3000 times during the index period), so slight changes are more likely to be captured by statistical tests.

To review or graph the continuous sampling data, select *Analysis and Export* from the *Main Menu*, and then select *Export to Excel*. From the list of available reports, highlight *Continuous WQ Data*. Click *Preview* to view the report, or *Export* to export the data.

2.13.2 *Submerged Aquatic Vegetation Reporting*

2.13.2.1 *Seagrass Mapping*

Producing maps that are thematically and geographically precise and accurate is very expensive, so measures are rarely replicated. All data have inherent errors; however, and this includes seagrass mapping data. Nevertheless, seagrass area is generally reported as a single value, and rarely are error terms included despite the fact that n=1 (Kurz 2000). Change analysis is complicated by the lack of a thorough understanding of the accuracy

and error associated with individual maps. This can make it difficult to interpret with rigor the small change between sequential maps. C-CAP mapping protocols instead rely on carefully constraining the errors, and evaluating them with quality assurance measures. Caution is advised when analyzing change to incorporate the best available estimates of these error terms.

Remapping of Seagrass habitat is prescribed for 5-year intervals. For each of these, the Network should use detailed maps to report the geographic distribution of SAV, areas of habitat increase, and areas of loss. For some of the mapping programs it will also be feasible to report on individual SAV cover classes. In addition to changes in areal extent, changes in frequency distribution among cover classes may illuminate interesting trends.

2.13.2.2 *Seagrass Condition Measures*

The objective of monitoring seagrass condition indicators is to compare trends in seagrass characteristics at different depths in existing seagrass habitat. The question of interest is whether changes in seagrass structural measures over time differ among the depth transects; we expect responses to nutrient enrichment to occur first in the deepest areas of seagrass beds, so significant differences in trends between deep and shallow transects may signify management problems associated with estuarine nutrient load.

The first step in analysis and reporting of seagrass condition measurements is to transform measured values (e.g. number of shoots counted in a 1/16 m^2 quadrat, biomass of vegetation collected within a 0.0035m^2 core) into standard, ecologically meaningful variables (e.g. density and biomass per m^2). The continuous variables of interest and their derivation are listed in Table 20, and sample calculations are provided in an attached MS Excel worksheet (Appendix 9).

Table 20. Derivation of seagrass condition variables.

Variable	Derivation
Total Shoots / m^2	(Shoots/0.0625 m^2)*16
Reproductive Shoots / m^2	(Reproductive shoots/0.25 m^2)*4
Canopy height (cm)	Direct measurement
Percent cover	Direct measurement
Total biomass (g/m^2)	(Total shoots/m^2) * (Total g/shoot), where g/shoot = (total g/core) / (number of meristems/core)
Aboveground biomass (g/m^2)	(Total shoots/m^2) * (Aboveground g/shoot), where Aboveground g/shoot = (Leaf + Sheath g/core) / (number of meristems/core)
Leaf biomass (g/m^2)	(Total shoots/m^2) * (Leaf g/shoot), where Leaf g/shoot = (Leaf g/core) / (number of meristems/core)
Ratio leaf:root+rhizome biomass	(Leaf g/core) / (Root+Rhizome g/core)
Ratio shoot:root+rhizome biomass	(Leaf + Sheath g/core) / (Root+Rhizome g/core)

Because data on seagrass condition indicators are collected from permanent sampling quadrats, consecutive samples are correlated. The advantage of using permanent

sampling quadrats lies in the power of the statistical tests used to detect change over time. Effectively, we are analyzing the *change* from one time period to the next at given sample locations rather than comparing mean conditions at each time period. The error for this test is the variation in the *change* between two time periods rather than the variation among sampling units at either time period (Green 1993, Elzinga et al. 1998). By eliminating the contribution of spatial variation among sampling units to experimental error, this approach results in a powerful ability to detect changes over time and a reduction in the number of sampling units needed to detect a certain magnitude of change.

To review or export the Seagrass Condition data both measured and derived, select *Analysis and Export* from the *Main Menu*, then select *Export Data to Excel*. From the list of available reports, highlight *Seagrass Condition - all indicator data*. Click *Preview* to view the table or *Export* to save it to an Excel formatted file.

The appropriate statistical approach to determining whether temporal patterns in seagrass condition measures differ among depths is a repeated measures analysis, with quadrats as the subjects of repeated sampling (Winer 1971). The design incorporates the between-subjects effect of depth and within-subjects main effect of time. Univariate repeated measures ANOVA assumes that all between-times correlations are the same (i.e., correlation between measurements of Year 1 and Year 2 is the same as between Year 2 and Year 3, Year 1 and Year 3, etc.) This assumption can be tested (Mauchley's criterion; Potvin et al. 1990) and if warranted, significance levels of the univariate test can be adjusted or a multivariate ANOVA can be applied (Potvin et al. 1990, Green 1993). Additional assumptions of both univariate and multivariate approaches to repeated-measures ANOVA are common to any parametric technique, namely normality and homogeneity of error variances (Green 1993). Residual analysis should be used to diagnose departures from these assumptions (e.g. Neter et al. 1990); should the data deviate strongly from these basic assumptions, the data must be transformed appropriately.

Annual reports should include graphs of mean seagrass response vs. depth station, or mean seagrass response vs. time at each depth station. These figures should be accompanied by results of the tests described above to determine whether annual changes in seagrass condition differs significantly among depths.

To report the averaged seagrass condition indicators from the Estuarine Monitoring Database, select *Analysis and Export* from the *Main Menu*, then select *Summary Reports*. From the list of available reports, highlight *Seagrass Condition - averaged by transect and year*. Click *Preview* to view the report or *Print* to print it.

To export a digital version of this data in Excel format for direct inclusion in a text document of for use in a spreadsheet or other program, select *Analysis and Export* from the *Main Menu*, then select *Export Data to Excel*. From the list of available reports, highlight *Seagrass Condition - averaged by transect and year*. Click *Preview* to view the table or *Export* to save it to an Excel formatted file.

Light, temperature, salinity, and sediment data should be presented as ancillary information. These habitat characteristics provide a framework within which to interpret measures of seagrass condition. Light intensity data collected by the HOBO loggers must

be merged into records of readings collected contemporaneously (i.e. as close to the same minute as possible) at the three stations (shallow, deep, and air). Information on the time of sensor launching, deployment, and retrieval (recorded on the Light and Temperature Sensor Deployment Log) must be used to crop all of the data logged before and after the period of deployment from the stored record. In addition, the submersible cases that house the underwater light sensors become coated with biofouling organisms during deployment, requiring judgment on how much of the 7-day data stream to retain. A network coordinator should make this decision based on the degree of difference between sensors in air and water; typically, a consistent increase in this difference indicates that fouling has begun to measurably limit light transmission to the underwater sensor. Finally, a file for analysis should be constructed of light data collected within two hours of local noon (however do not discard data outside this analytical window). The following derived variables should then be calculated on this subset of light data for the period from initial deployment to the chosen end date:

light intensity at the shallow station (A) as a percentage of incident light: ----- A0/Air*100
light intensity at the deep station (C) as a percentage of incident light: --------- C0/Air*100
Attenuation coefficient (K_d) of downwelling irradiance:---------- ln (C0/A0)/-z

where A0, C0, and Air represent light intensity logged at the respective stations, and z is the difference in depth between station A0 and C0 in units of meters measured for that monitoring interval. These values should be averaged for each depth station over the monitoring interval.

The Estuarine Monitoring Database includes developed queries and routines to match the light sensor data across the stations. Once these are correlated, the analysis has been automated in the database already. Select *Seagrass Condition – light intensity data* from either the export or the reporting menu.

Temperature data should be similarly cropped to eliminate data outside the period of deployment from the stored record. Temperatures should be plotted as logged (i.e. there are no derived variables) over time for each depth station. Salinity should be reported as individual measurements at each depth station, and sediment characteristics reported as means for each depth station at each monitoring interval.

To review or export the temperature data, select *Analysis and Export* from the *Main Menu*, and then select *Export Data to Excel*. From the list of available reports, highlight *Seagrass Condition – temperature log*. Click *Preview* to view the table or *Export* to save it to an Excel formatted file.

Similarly, to review or export the salinity and sediment data, select *Seagrass Condition – salinities* or *Seagrass Condition – sediment data*, respectively, from the *Analysis and Export* menu. Click *Preview* to view the table or *Export* to save it to an Excel formatted file

2.13.3 *References*

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2.14 SOP 14 – Using a Garmin V GPS Unit (see link below)

http://www1.nature.nps.gov/im/units/ncbn/products/Data_manage/NCBNAppendix_GarminVSOP_Final_2004Dec5.pdf

2.15 SOP 15 – Revising the Protocol / Version Control Procedures (to be developed)

3 APPENDICES

3.1 Appendix 1: Excel Spreadsheet: Sampling Point Locations Database

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A1.sampling_pt_db.xls

3.2 Appendix 2: 13th Coast Guard District Private Aids to Navigation Information Handout

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A2.PAToN_handout.pdf

3.3 Appendix 3: 13th Coast Guard District Private Aids to Navigation Application

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A3.PAToN_application.pdf

3.4 Appendix 4: YSI-LiCor Sonde Calibration Log

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A4.YSI-LiCor_Calibration_Log.pdf

3.5 Appendix 5: YSI Chlorophyll Sensor Calibration

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A5.YSI_Chl-a_calibration.pdf

3.6 Appendix 6: Spatial Survey Data Sheet

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A6.Spatial_Survey_Data_Sheet.pdf

3.7 Appendix 7: Excel Spreadsheet: Chl-a Filtering Log

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A7.Chl-a_filtering_log.xls

3.8 Appendix 8: Excel Spreadsheet: Submerged Aquatic Vegetation Monitoring Field Sheets

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A8.SAV_monitoring_field_sheets.xls

3.9 Appendix 9: Excel Spreadsheet: Seagrass Condition Template

http://www.nature.nps.gov/im/units/ncbn/products/Monitoring/Estuarine_Nutrient_Protocol/Appendices/A9.Seagrass_Condition_Template.xls